THE PHYSIOLOGY OF VASODILATATION*

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INTRODUCTION

In undertaking this paper, no attempt has been made to include a comprehensive bibliography of the copious literature on the subject. Rather an effort has been made to hold references to a minimum, citing only that literature which represents a major advance to the knowledge and understanding of the subject of reflex dilatation.

Since the terms active and passive dilatation will be used frequently in this paper it is perhaps appropriate at the outset to state the context in which these terms are used. Passive dilatation refers to the vascular relaxation which occurs when tonic secretion of a constrictor substance is interrupted. The existence of neurogenic tone is therefore a prerequisite if passive reflex dilatation is to occur. It also follows as a corollary that the maximal possible amount of passive reflex dilatation is determined by the amount of existing tone. Active dilatation refers to the vascular relaxation following the endogenous release or the exogenous administration of a substance capable of decreasing tone by a direct action on the blood vessel. In contrast to passive dilatation which is brought about by a decrease in constrictor substance, active dilatation reflects an increase in vaso-active substance at the blood vessel level. Active dilatation also differs from passive dilatation in that neurogenic constrictor tone is not a prerequisite for active reflex dilatation to occur. It is only necessary that vascular tone be present irrespective of whether it is neurogenic, humoral, or intrinsic in origin. The magnitude of active dilatation, on the other hand, is determined by the extent of total vascular tone. In rare instances, such as the intra-arterial injection of serotonin, dilatation may not occur without an intact sympathetic supply even though the tone remains largely unchanged.

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REFLEX MECHANISMS AVAILABLE FOR THE PRODUCTION OF PASSIVE REFLEX DILATATION

In the past few years there have been several excellent reviews on this subject. The papers by Dawes and Comroe[30] and by Aviado and Schmidt[5] summarize most of the pertinent literature and critically integrate the findings of the numerous investigators. The more recent monograph of Heymans and Neil[58] deals expansively with the subject of buffer mechanisms, its historical development, its anatomic basis and its physiologic integration. Several other recent sources provide valuable additional information and discuss the concepts involved.[7, 28, 37, 46, 76, 94, 111, 113,114] To re-state the work contained in these recent authoritative reviews would only be repetitive. Instead a summary of passive reflex mechanisms and their pathways will be presented for the more facile understanding by those students whose special interests lie in more peripheral areas. The dedicated student with deep-rooted and enduring interest in the delicacies of autonomic vascular control is referred to the above sources or to the original literature for a comprehensive and critical coverage.

The most well known and probably the most important mechanisms capable of producing passive reflex dilatation are the carotid sinus and aortic arch buffer systems. In both of these regions are located specialized afferent receptors which respond either to vascular distension or to stretch. The stretch initiates a volley of impulses which pass centrally over the sinus or vago-depressor branch of the vagus to inhibit tonic discharge of the vasomotor center. The inhibition of vasomotor activity results in the passage of fewer sympathetic impulses to the peripheral blood vessels. Consequently, less norepinephrine is released from the nerve endings; that norepinephrine previously discharged is quickly terminated by enzymes present in the vessel, the free norepinephrine concentration quickly falls, and the vessel relaxes.

The existence and importance of these reflexes are well documented. The contention that these reflexes are responsible for the primary control of

blood pressure is supported by the well substantiated observations that carotid occlusion results in a reflex rise in blood pressure, that combined sinusectomy and vagotomy result in a marked and sustained rise in blood pressure, and, conversely, that maneuvers such as increasing the sinus pressure or painting the sinuses with norepinephrine, or electrically stimulating the sinus nerves result in reflex hypotension. These observations lead to the concept that the vasomotor center has an inherent tendency to discharge at a high level of activity but is held in check by the inhibitory input from these pressure sensitive zones. The injection of a pressor substance, by elevating the blood pressure, would increase the inhibitory input from such areas and, by decreasing the secretion of endogenous catechol amines, would result in passive reflex dilatation.

Several other reflexes have been described which produce a reflex lowering in blood pressure and which are assumed to operate as vasomotor inhibitory systems. Two of these, the pulmonary depressor reflex and the coronary chemoreflex (part of the Bezold-Jarisch reflex) are initiated by chemical stimulation rather than by stretch. The afferent receptors of the former reflex are located in the distribution of the pulmonary artery. The afferent receptors of the coronary chemoreflex are in the distribution of the coronary artery and the pulmonary vein, the relative contribution from these sources being species dependent. Impulses initiated from these areas by specific chemical stimuli pass centrally over the vagus. The central distribution and mechanism of action of these reflexes has been assumed to be similar to those arising from the sinus and aortic arch; that is, that these reflexes also operate to produce passive reflex dilatation through inhibition of sympathetic tone.

It has long been recognized that afferent splanchnic stimulation can produce a reflex fall in blood pressure.[21, 22] Gruhzit and associates[56] demonstrated that epinephrine, and to a lesser extent norepinephrine, is capable of producing a reflex dilatation in the cross-circulated hindlimb of the dog after sinusectomy and vagotomy. Gruhzit and his colleagues proposed that the receptors for the reflex were located along the distribution of the aorta and coined the name mechanoreceptors, because they felt that the receptors discharge in response to pulse pressure. They reported that the reflex response to epinephrine after sinusectomy and vagotomy was abolished by section of the dorsal thoracic roots. Recent interest has been aroused in the functional role of receptors located in the mesenteric distribution. Sarnoff and Yamada[93] believe that such receptors may tonically influence the blood pressure of the cat. Heymans and his associates[57] have taken exception to this view and feel that the response can be explained as an artifact of the experimental conditions. Recently, however, Selkurt and Rothe[100] have also investigated this problem. These authors feel that the reflex is of little consequence in the dog but feel that it is of more biologic significance in the cat. The fact that sinusectomy and section of afferents arising from the aortic and pulmonary vessels results in a sustained rise in blood pressure which may last for months or longer questions the importance of such reflexes in the long term regulation of blood pressure. They may well be important in short term regulation of blood pressure, such as in exercise, shock, and so on, or they may play an auxiliary or secondary role in blood pressure regulation. They may well account for the fact that the blood pressure of the neurogenic hypertensive animal ultimately returns toward normal levels.

A centrally located system capable of producing reflex dilatation and excited by epinephrine has been reported by Taylor and Page.[106, 107] Upon re-investigating this problem Schneider and associates[97] reported only minimal hypotensive responses in the recipient body when epinephrine was injected into the sinusectomized cross-circulated head. Because of the large dose of epinephrine required to produce the minimal hypotensive response they question the physiologic importance of such a reflex mechanism.

The level of sympathetic activity in relation to passive dilatation and its clinical significance. The therapeutic use of a large number of pharmacologic agents is based on their ability to produce passive dilatation. Agents are available which initiate the Bezold-Jarisch reflex, depress the vasomotor center by elevating threshold, block sympathetic ganglia, prevent the release of norepinephrine from the sympathetic nerve endings, and block the vasoconstriction produced by released norepinephrine. Because all of these agents can only produce passive dilatation, sympathetic tone must be present if they are to

be clinically effective. Until recently it was generally accepted that the normal individual or animal possessed considerable sympathetic tone even under basal conditions. This concept has been questioned. Maxwell and associates[84] reported that the administration of the ganglion blocking agent, chlorisondamine, failed to lower pressure in normal unanesthetized dogs and rats, and inferred from this observation that sympathetic tone was absent in these species without anesthesia. A well defined fall was observed in the unanesthetized monkey and rabbit, however. Similar conclusions that sympathetic tone is absent or minimal have been drawn from the observation that small doses of ganglion blocking agent, capable of lowering the standing blood pressure in hypertensive patients, fail to lower materially the supine blood pressure. The same observation has been made with relatively small doses of some of the new norepinephrine antirelease agents such as bretylium and guanethidine.

If the above observations could be taken as inarguable evidence of the absence of sympathetic tone in the resting state, it would have important implications and consequences. In hypertension, for example, it would necessarily implicate a non-neurogenic component as the responsible factor for the elevated pressure. This in turn would point up the futility of using agents which produce passive dilatation to attempt to lower the blood pressure of the sleeping or bed-ridden hypertensive patient. If the passive dilating agent were able to lower only the standing blood pressure, it would indicate that the sympathetic tone of the hypertensive patient had increased so as to prevent a fall in standing pressure. This would favor the proposal that the barostat or pressure regulating mechanisms are set at a higher level in the hypertensive patient.

Not all researchers are in agreement, however, that sympathetic tone is virtually absent in the resting state. In anesthetized animals, where it is most easy to evaluate sympathetic activity, it is almost universally agreed that a large complement of sympathetic tone exists. But it is entirely possible, and in fact proposed by many, that anesthesia calls forth an abnormal complement of sympathetic tone. In an unanesthetized dog, immobilized by decamethonium, and artificially respired, Beck[10] noted a large reduction in cardiac output and a considerable fall in blood pressure following large doses of ganglion blocking agents. In some experiments currently being carried out, Dr. Donald Knapp of this department, has observed a fall in blood pressure after 4 mg per kg of ostensin or 5 mg per kg of hexamethonium in unanesthetized cats and dogs immobilized with decamethonium. Mid-pontine section often produced a further fall in blood pressure following ganglionic blockade. Ganglion blocking agents produced a fall in blood pressure in animals with a mid-pontine section. The section was carried out under ether anesthesia. The ganglion blocking agents were administered 2 hours after anesthesia to allow the ether to blow off. It is noteworthy that the pressure responses to the ganglion stimulating agents, nicotine and dimethylphenylpiperazinium, were almost totaly abolished by ostensin, whereas auditory and visual stimuli were still capable of evoking sharp rises in blood pressure in non-transected animals but these responses were not long maintained. These latter responses occurred more rapidly than could be accounted for by adrenal discharge.

Laverty and Smirk[74] found that the increase in hindlimb resistance in spontaneous and renal hypertensive rats is neurogenically maintained in that it could be blocked by hexamethonium. Hexamethonium reduced the blood pressure of normal, spontaneous and renal hypertensive rats to less than half, but the floor blood pressure of the renal hypertensive animals was greater than that of the other two groups. Using his indwelling aortic catheter technique[116] Dr. James Weeks (unpublished observations) of the Upjohn Company has also observed a large fall in blood pressure following administration of large doses of hexamethonium (10 mg per kg) and tetraethylammonium (20 mg per kg) in unanesthetized renal hypertensive rats. The floor pressure observed by Weeks was considerably higher than that observed by Laverty and Smirk.[74] Interestingly, doses of 5 mg per kg and 10 mg per kg of tetraethylammonium appeared to have little effect on blood pressure either in the unanesthetized or anesthetized hypertensive rat. These observations suggest that some types of constrictor activity are remarkably resistant to low doses of ganglion blocking agents but can be blocked by very large doses. It is possible that the hypotension is brought about by other than neurogenic blockade, but this seems doubtful since hexamethonium may increase the blood pressure of the unanesthetized spinal cat, and

has little effect on the blood pressure of dogs whose spinal cord is perfused with local anesthetic from the lumbar to cervical areas (personal observations). The findings of many investigators are thus not in accord with those of Maxwell and associates[84] who felt that tonic sympathetic activity to the blood vessels did not exist in the unanesthetized dog and rat. It raises the question of whether adequate dosage of blocking agent had been given to block sympathetic tone to the blood vessels.

In the human there is also some evidence of tonic sympathetic activity in essentially basal states. The patient at bed rest continues to secrete catecholamines in his urine. High spinal anesthesia generally produces a fall in blood pressure even in the supine state. Neurosurgeons often have difficulty maintaining the blood pressure immediately following sympathectomy.

These observations are clearly in opposition to the view that sympathetic tone is virtually absent in basal states. From a clinical standpoint, an evaluation of the amount of existing sympathetic tone is of fundamental importance. It dictates to a very large extent whether agents capable of producing passive dilatation through blockade of neurogenic tone are to be used, or whether agents capable of producing active dilatation by direct or reflex means are to be used.

ACTIVE DILATATION

As stated previously, the basis for passive dilatation is well founded experimentally and generally accepted. Active reflex dilatation is neither as well founded nor is it so generally accepted. Because of the more general controversial nature of the latter area and because of the sometimes problematic interpretations put upon data by various investigators, more space will be devoted to the discussion of this subject.

The concept of a vasodilator center. It is interesting that the same investigator, Claude Bernard,[12, 13] was not only the first to demonstrate passive dilatation by his classic rabbit ear experiment, but he was also the first to show active dilatation in the submaxillary gland upon stimulation of the chorda tympani nerve. This observation quite naturally led many early workers to assume the existence of a higher center which governed active dilatation as well as a vasoconstrictor center which governed vasoconstriction. Some of the early workers maintained that only a vasoconstrictor center existed and that vasodilatation resulted from inhibition of tonic vasoconstrictor activity. Thus the controversy which still exists today was hotly contested before the turn of the century.

The early work relating to a vasoconstrictor and vasodepressor center was reviewed in a monograph by Bayliss in 1923.[9] A few significant observations relating to the early development of these concepts deserve special mention. Schiff in 1855[95] measured the temperature of the dog paw before and after transection of the medulla at various levels and found the level of the obex to be critical in the maintenance of paw temperature. Shortly after in 1873, Dittmar[31] made successive brain sections from above downward in curarized rabbits and observed no effect on blood pressure until the mid-pontine level was reached. From this point to a level just above the obex, successive sections resulted in a progressive fall in blood pressure. Sections below the obex produced no further fall in pressure. Ranson and Billingsley in 1916[87] explored the floor of the fourth ventricle for vaso-active areas producing a fall or rise in blood pressure. They noted an area in the inferior fovea which produced a marked pressor response and a discrete region near the area postrema which produced a marked depressor response. They also noted that stimulation of other areas led to less pronounced blood pressure changes, but it was much later when Wang and Ranson in 1939[116] and Alexander in 1946[1] emphasized the diffuseness of the vasopressor and vasodepressor areas in the brainstem.

In the early phase of the investigation into the existence of separate vasopressor and vasodepressor centers, much emphasis was placed on the observation that afferent stimulation of most peripheral nerves would produce depressor responses at low intensities of stimulation and pressor responses at greater intensities. In an excellent deductive research, Ranson and Billingsley[88, 89] were able to show that the afferent pathways for the depressor and pressor reflexes ascend in different parts of the spinal cord. A separation of the central input of the vagodepressor reflex and the somatic nerve reflex was reported by Langley in 1912[73] who injected starch into the internal carotid artery which apparently produced selective infarction. The role of the pressor and depressor areas in the integration of vasomotor reflex activity was

initially examined by Scott and Roberts in 1923[98] and by Scott in 1925.[99] Scott abolished the reflex hypotension deriving from central stimulation of the vagus by cauterization of the medullary depressor areas of Ranson and Billingsley. He found that this procedure left unaffected the reflex hypotension evoked by stimulation of the central end of the brachial or sciatic nerves. These observations have been recently confirmed for vagus and sinus nerve stimulation and left posttibial nerve stimulation by Lindgren and Uvnas in 1954.[81] Scott concluded from his observations that the depressor area was not the "supreme vasodilator center" in that not all vasodilator reflexes pass through it.

In more recent years much evidence has been accumulated that stimulation in many parts of the brain above the medullary area can produce a rise or fall in blood pressure depending on the stimulated area, the anesthetic state, and the physiologic status of the animal. In most cases, however, it is not possible to determine whether the fall in blood pressure initiated by stimulation is active or passive since most often only blood pressure changes have been measured. Because the blood pressure bears no necessary relationship to the resistance of the blood vessels as a whole and particularly no relationship to the resistance in individual vascular beds, interpretation of the results in most cases must await further experimentation. At the moment the only definitive evidence for active dilatation initiated by central stimulation derives from the work of the Scandinavian workers which is discussed in more detail below.[33, 34, 45-51, 77-81, 111-113] The current status of the central nervous control of the circulation has been presented in a recent symposium.[23]

Although much of the work dealing with higher center control remains obscure, there is, nevertheless, considerable evidence for neurogenically mediated active dilatation. The dilatation obtained by stimulation of the decentralized dorsal roots (antidromic stimulation) has long been recognized and has been the subject of rather intensive investigation. Because histamine has been implicated in this form of dilatation, it is covered under the section of the Role of Histamine in Neurohumoral Transmission. In more recent years interest in the subject of active dilatation mediated by neurogenic means has been stimulated by the observations of Folkow, Uvnäs and their collaborators[33, 34, 45-51, 77-81, 111-113] following their discovery of active dilatation in the hindlimb of the cat produced by stimulation in various areas of the brain. Since the weight of evidence indicates that this system is cholinergically mediated it is discussed in more detail under The Sympathetic Cholinergic System. Impetus was also added by the discovery of Barcroft and associates[6] that active reflex dilatation occurred during fainting. These workers observed that on fainting the dilatation occurring in the forearm of a patient with an intact sympathetic supply was greater than the dilatation in the forearm in which sympathetic transmission had been interrupted. The mediator of this type of dilatation has not been worked out. The literature pertaining to fainting has been reviewed by Barcroft and Swan.[7]

Reflex dilatation initiated by pressoreceptor mechanisms has been generally considered to be passive in character. In the excellent review by Heymans and Neil[57] the authors concluded that baroreceptor vasomotor reflexes do not activate the sympathetic vasodilator nerves (page 43) and cite as evidence papers by Folkow and Uvnäs, Lindgren and Uvnäs, and Frumin, Ngai and Wang. The Scandinavian workers have clearly shown that sinus reflexes are not involved in cholinergic vasodilatation mediated over the sympathetics since reflex dilatation induced by sinus stimulation was not blocked by atropine.[51, 81] These authors also excluded dorsal root contribution in the sinus or vagodepressor induced reflex dilatations because they had previously ligated the spinal medulla at L-5.[81] They assumed that the baroreceptor induced type of reflex dilatation was therefore passive in nature. Folkow and Uvnäs[51] reported that dibenamine blocked reflex dilatation produced by physiologic saline administration. This observation would not necessarily rule out histamine as a mediator (see below) since dibenamine has been reported to prevent the release of histamine.[68] It also has antihistaminic actions,[55] although not as potent as dibenzyline which also blocks this type of reflex dilatation to a variable degree.

Frumin, Ngai and Wang[52] investigated reflex dilatation in the cross-circulated hindlimb of the dog induced by afferent vagal stimulation and by de-occluding the previously restricted blood supply through the carotid arteries. The

reported that large intra-arterial doses of hydergine or ganglionectomy abolished the reflex dilatation. Hindlimb dilatation induced by lumbar sympathetic stimulation could be abolished by hydergine and large doses of atropine. They concluded that reflex dilatation induced by the above means was passive and reflected an inhibition of the sympathetic vasoconstrictor activity. This paper is widely cited as evidence for the absence of active dilatation induced by baroreceptor mechanisms and therefore deserves special attention. Careful perusal of their records suggests that active reflex dilatation did indeed occur in their experiments. Attention should be focused on figures 3, 4 and 7 of their paper. In figures 3 and 7 reflex dilatation induced by afferent vagal stimulation and release of the carotids, respectively, is clearly blocked by intra-arterial hydergine as the authors have stated. But the post-blocking blood flow to the limbs, however, does not nearly approximate the blood flow induced during the reflex, which it should have done, were the increase during the reflex the simple resultant of having removed vasoconstrictor tone. In both of these records the blood flow is two- to three-fold greater during the reflex than it is after blockade of the reflex. The failure of blood flow to increase following hydergine might be attributed to a direct counter vasoconstriction by hydergine but this mixture does not normally produce a very marked constriction as compared to the large vasoconstricting action of some of the other ergot alkaloids. The fact that adrenergic blockade was induced by these large intra-arterial doses of hydergine may well be misleading for as will be seen later we have blocked reflex dilatation with intravenous doses which were only about $\frac{1}{50}$ the intra-arterial dose used by these investigators. At these small dose ranges significant adrenergic blockade does not usually occur. Further, reflex constriction induced by several means is not impaired at these doses in the dog nor is constriction produced by direct sympathetic stimulation. In the paper by Frumin and associates,[52] an even clearer demonstration of active dilatation, without the complicating factor of drug intervention, is provided in the last three panels of their figure 4 after the artifactual effects of collateral blood flow had been obviated in the preparation. Initiating the reflex produced a three- to six-fold increase in the resting blood flow of the innervated limb but failed to increase the blood flow in the denervated limb. After collateral occlusion the resting blood flows are similar in both the innervated and the denervated limbs indicating that sympathectomy had produced no very evident release from vasoconstrictor tone, certainly not a three- to six-fold increase. It therefore appears that active dilatation primarily accounted for the increase in flow in the innervated limb which was prevented in the denervated limb by the previous sympathectomy. Thus three of the illustrations by Frumin, Ngai and Wang suggest the presence of active reflex dilatation in spite of their conclusion that it does not occur. Their results, if not their conclusions, are thus in accord with our own observations cited below as well as those of the French workers, Binet and Burstein.[14, 15] The latter authors also used the cross-circulated hindlimb preparation of the dog and evoked reflex dilatation by the administration of epinephrine to the recipient body. They concluded that active reflex dilatation must occur because the reflex dilatation exceeded the passive dilatation produced by sympathectomy of the area. They failed to block the reflex by administration of atropine or to potentiate it by physostigmine, an observation which is consonant with the later findings of Lindgren and Uvnäs that cholinergic fibers are not involved in this type of reflex.[81]

It becomes evident, therefore, that many of the investigations involving baroreceptor reflexes and carried out in independent laboratories yield very similar results. The interpretations of the results are often at variance, however. In some cases the conclusions appear to be unjustified because of the failure to consider other than classical or well known actions of the pharmacologic tools utilized in the various investigations.

Gruhzit, Freyberger, and Moe[56] demonstrated that epinephrine can produce reflex dilatation in the dog hindlimb following sinusectomy and vagotomy. They did not attempt to determine whether this reflex was active or passive in nature and the question still remains to be answered.

Recently Litwin, Dil and Aviado[82] showed that active reflex dilatation occurs in the perfused hindquarters of the dog in response to hypoxia.

The response remained after treatment with bretylium and was abolished by sympathectomy. It appeared to be partially blocked by atropine. In our own studies cited below we had observed an active reflex dilatation following a period of tracheal occlusion which is very likely the same phenomenon reported by Aviado and his colleagues. In a pre-publication communication with the above authors, we indicated that we also felt the active component was partially blocked by atropine. We are now no longer sure whether the apparent partial blockade by atropine is specific or non-specific (see later).

Shaw and associates [101, 102] reported that the dilator component of the biphasic blood pressure response produced by large doses of acetylcholine in the atropinized animal can be blocked by nicotine and curare. They felt that the dilator component was not due to an override of the atropine blockade and theorized that two types of ganglion cells are present which they refer to as C (constrictor) and D (dilator) cells. A recent paper by Gardier and associates[54] would seem to support the view that the depressor component is a form of active dilatation. The pressor component could be essentially abolished by administration of *N*,*N*-diisopropyl-*N*-isoamyl-*N*-diethylaminoethylurea. More definitive experiments will be required to answer this problem. It is interesting that the latter authors were not able to block the dilatation by diphenhydramine, and also discount an override of cholinergic blockade induced by atropine. The nature of the dilatation remains obscure.

The active dilatation produced by exercise will not be discussed in this paper.

There thus exists in the literature a considerable amount of evidence for the existence of active dilator mechanisms. The nature of the substances mediating these active dilator mechanisms will now be discussed.

The sympathetic cholinergic system. In a classic paper on ergotoxine by Dale[29] the "motor" effects of stimulation of sympathetic nerves were found to be either abolished or reversed by the administration of the ergot alkaloids. With regard to the vasculature it was often found that ergotoxine converted sympathetic constriction to a dilatation. Dale concluded that vasodilator fibers exist in the sympathetic chain whose effects are masked by the "motor" components of the complex nerve. The Rogowicz phenomenon (stimulation of the cervical sympathetic chain following motor denervation producing contraction and flushing of the lips) was studied by Euler and Gaddum (1931).[40] These investigators felt that the fibers causing the flushing were identical with those responsible for the motor response. The phenomenon was believed to be due to the release of acetylcholine from sympathetic nerves. Burn[18, 10] studied the vascular responses to stimulation of the abdominal sympathetics in the perfused hindend of the eviscerated dog. Vasodilatation, vasoconstriction, or a combination of the two responses was elicited, dependent upon the parameters of stimulation. Intense stimulation of short duration often gave rise to dilatation, whereas prolonged stimulation usually resulted in vascular constriction. These effects were heightened by the infusion of epinephrine previous to the application of the stimulus. Other variations seen by Burn were an initial dilatation followed by constriction, and a dilatation following constriction upon cessation of stimulation. These results were confirmed by Schneider.[96] Bülbring and Burn[16, 17] demonstrated the cholinergic nature of the sympathetic vasodilators. In perfused hindquarters of cats and dogs dilator responses were potentiated by eserine pretreatment and abolished by atropine. Venous effluent of Ringer's perfusate collected during sympathetic stimulation was found to contain acetylcholine when bioassayed on the leech preparation. Similar evidence for sympathetic cholinergic vasodilatation was obtained simultaneously and independently by Rosenblueth and Cannon.[9] Vasodilatation induced in hindlimbs of cats by stimulation of the sympathetic chain following dibenamine was also shown to be cholinergic by direct assay of venous effluent.[51] The fall in blood pressure in cats produced by the effluent was abolished by atropine and potentiated by eserine.[49] Bülbring and Burn[17] were unable to demonstrate sympathetic vasodilatation in the skin of the dog either before or after ergotoxine. The selective distribution of these fibers to the skeletal muscle has more recently been confirmed by Folkow and Uvnäs.[51] Evidence that sympathetic vasodilatation exists in the ear skin of the dog has been challenged.[17] Folkow and co-workers argued that the plethysmographic technique utilized by Bülbring and Burn was not specifically measuring skin volume

but rather included a significant quantity of skeletal muscle at the base of the ear.

The sympathetic vasodilators were shown to be true post-ganglionic sympathetic nerves.[51] Typical cholinergic dilator responses were obtained in cats following lumbar dorsal root section and were abolished by pre-ganglionic degeneration.[81]

During the past decade, the central nervous system representation of the sympathetic vasodilator system has been investigated in some detail. A pathway from the motor cortex to the spinal cord has been rather specifically traced utilizing the Horsley-Clarke technique, histologic identification of positive dilator areas, and cholinergic blockade with suitable low doses of atropine.[77–80]

Eliasson and associates[33, 34] were able to evoke cholinergic vasodilator discharge by stimulating an area in the motor cortex near the cruciate sulcus. The efferent projections from this area pass caudally to the hypothalamus at which point there is apparently a neuronal relay. Evidence favoring a neuronal relay derives from the experimental observation that cholinergic dilatations can be produced at the hypothalamic level in chronic decorticate animals. At the posterior end of the hypothalamic area, the tract passes dorsally to the collicular area, where another neuronal relay has been identified by medullary stimulation in chronically supracollicularly decerebrate dogs. From the collicular area the pathway turns ventrally and passes through the ventrolateral portion of the medulla to the lateral horn of the spinal medulla. The authors state that cross-circulation of the hindlimbs has in all of the above-mentioned investigations been used to establish definitely the peripheral neurogenic nature of the dilator system. Moreover, Folkow and Gernandt[48] observed increased activity of an electroneurogram from the peroneal nerve upon excitation of the hypothalamic cholinergic vasodilator area. Suprabulbar stimulation resulted in bilateral hindlimb dilatation, whereas medullary stimulation produced the typical response in the ipsilateral limb only.

In summary, a cholinergic vasodilator system with extensive central representation has been demonstrated in cats and dogs. Excitation of this system results in active dilatation of skeletal muscle vasculature. The physiologic significance of the sympathetic cholinergic dilators is unknown.

Polypeptides and reflex dilatation. Several vasoactive polypeptides have been known to exist for a number of years. Advances in chemical techniques have permitted the synthesis of vasopressin, oxytocin, angiotensin and bradykinin. Following synthesis it has been found that extremely small quantities of these compounds are capable of producing vasoconstriction or vasodilatation depending on the compound. The marked activity of these compounds naturally raises the question of whether they are involved in reflex mechanisms governing the vasculature. Although vasopressin may be liberated by afferent vagal stimulation or asphyxia it is presumed to be released from the posterior pituitary or neighboring structures.[25] There is no evidence as yet that it is reflexly released from other areas. The primary evidence to date is that angiotensin is formed from plasma proteins by the action of an enzyme renin which is released from the kidney or possibly from other sources. It is generally presumed that the release of renin is not dependent upon neurogenic influences but the observations of Taquini and associates[105] that spinal pithing lowers the blood pressure of the renal hypertensive rat to the same level as the spinal pithed normal rat raises an interesting question in this regard. (Compare these findings to those of Laverty and Smirk,[74] and Weeks, unpublished observations, cited previously.)

A great deal of work has recently been carried out on the kinins. The subject has been recently reviewed by Lewis.[76] At present it is believed that the kinin-forming enzymes exist as inactive precursors which when activated, form kinins from plasma globulin. Whether there are a large number of kinins or perhaps only one is not yet known. Most work has been done with the kinin known as bradykinin which has been recently synthesized and found to be a nonapeptide. The preliminary pharmacology carried out with this substance suggests that it is identical with the natural bradykinin.[71] The recent interest in the possibility that bradykinin is important in vasoregulation derives primarily from studies of Hilton and Lewis[59–63] who showed that kallikrein could be obtained from the venous effluent and the secretions of the salivary gland after stimulation of the chorda tympani. The question which remains to be satisfactorily

answered is whether an enzyme, released by nerve stimulation, can produce kinin rapidly enough to dilate effectively the vessels in the area in which it is released. This consideration is of less importance when considering relatively static blood flow states such as those required to produce reactive hyperemia, but is a very important consideration from the standpoint of whether such a mechanism could operate where maximal reflex dilatation is produced in a very brief period of time. Direct release of a preformed dilator would seem much more appropriate under the latter circumstances. In this connection, Hilton and Lewis were not able to find a plasma kinin nor a quick-acting kinin forming enzyme upon antidromic stimulation to the perfused skin of the cat hindlimb. They also failed to detect kinin upon stimulation of the nerves to gastrocnemius muscle. Thus while kallikrein can be found in many glandular tissues, in the blood and in the urine, the physiologic role of the kinins as vascular regulating agents remains to be fully established.

Another polypeptide known as substance P was first isolated by Euler and Gaddum.[39] This substance is also a strong vasodilator. The exact potency is unknown since as yet it has never been synthesized. It is found in the gastrointestinal tract and in many areas of the brain.[53] As yet it has not been implicated as a neurogenically released dilator.

An excellent and well referenced coverage of the literature pertaining to the polypeptides can be found in the proceedings of a symposium on polypeptides edited by Dr. M. Schacter.[94] Lipoproteins have been reviewed by Vogt.[114]

The role of histamine in neurohumoral transmission. Barger and Dale[8] demonstrated that histamine was a normal constituent of body tissues. It is now well accepted that histamine exhibits an unusually wide distribution; only bone and cartilage have not been examined for their histamine content, but all other body components do contain the amine although in widely varying quantities.[44] The variation in organ content from species to species is also a factor that has been considered in studies on histamine distribution.[35, 36, 41, 117]

The ubiquity of this potent dilator material has prompted many investigations into determination of a possible role of histamine as a vasodilator in various physiologic and pathologic occurrences.[75] Among these may be included the anaphylactic reaction, headache, the "triple response" of Lewis, antidromic vasodilatation, post-occlusion hyperemia, functional hyperemia of exercise and gastric secretion. In this discussion emphasis has been placed on the considered role of histamine as a chemical mediator between nerve and effector cells.

Kwiatkowski[72] was the first to demonstrate the presence of histamine in nervous tissue. He reported "high" levels in dorsal root fibers. Coujard[27] utilized a mercury precipitation histologic technique claimed to be specific for histamine and found "histaminergic" nerves in Auerbach's plexus. The distribution of histamine in autonomic nerve fibers was examined in considerable detail by Euler[35, 36] and by Rexed and Euler.[90] Post-ganglionic fibers of the sympathetic system were found to possess the highest concentrations of histamine when compared with other autonomic nerves.[36, 118] The splenic nerve of oxen was found to contain the highest quantity averaging approximately 100 μg per gram of tissue. An attempt was made to correlate histamine and noradrenaline content of post-ganglionic sympathetic fibers.[41] In several species, those nerves rich in catecholamine were often found to contain also the highest concentration of histamine. However, wide variations in histamine to noradrenaline ratios were observed between the species studied (sheep, dog, cat, rabbits, oxen and guinea pigs). Similarly both histamine and noradrenaline concentrations were dominant in nerves possessing unmyelinated fibers, anatomically the post-ganglionic fibers of sympathetic origin. The well known correlation between the noradrenaline content of a sympathetic nerve and its organ of innervation does not hold for histamine. For example, in oxen, the splenic nerve and the post-ganglionic nerves to the ileum contain 60 to 100 μg per gram of histamine whereas the spleen contains 6 to 10 and the ileum 80 to 100 μg per gram. Unlike the noradrenaline content of organs which falls following the loss of sympathetic innervation, organ levels of histamine apparently do not follow this pattern. This phenomenon has not been studied in the same detail for histamine as it has for norepinephrine. Of the three denervated structures studied in sheep (spleen, salivary glands, and kidney) the histamine content of only the spleen was found to decrease.

The investigations of Werle and co-workers on the histamine content of nerves have been reviewed by Werle.[118] Werle's results in cattle differ from those of Euler only in a quantitative manner; ganglia and post-ganglionic fibers of the sympathetic system exhibited the highest titer of histamine. Of special interest is the demonstration by those workers of histidine decarboxylase activity in autonomic fibers. This activity, although of small magnitude, was much greater in sympathetic trunk material than in vagus nerve, which in turn was considerably higher than brachial plexus and sciatic nerve. The enzymatic conversion of histidine to histamine was considerably augmented by the addition of pyridoxal-5′-phosphate.

Euler and Aström[38] reported release of histamine from isolated splenic nerve of cattle by electrical stimulation. The isolated nerves were either stimulated in Ringer's which was subsequently transferred to a bath containing a strip of guinea pig ileum, or the cut end of the nerve was stimulated while submerged in the gut bath. Positive responses were obtained in both cases which were obliterated by antihistamine. It has not been claimed by the authors that this observation bears any necessary relationship to histamine release from nerves under normal physiologic conditions.

The question of whether or not histamine mediates the vasodilatation observed upon antidromic stimulation of dorsal root fibers remains a highly controversial one. Lewis and Marvin[75] proposed that a histamine-like mediator was released during antidromic stimulation of fibers from cat paw. The hypothesis was based entirely on indirect evidence, such as the prolongation of reactive hyperemia by antidromic stimulation attempted during arterial occlusion. Kibjakow[69] noted that venous effluent collected from cat hindlimb during faradization of dorsal roots contained a stable material which dilated the vessels of the rabbit ear. Kwiatkowski[72] observed that this substance apparently could be neither acetylcholine, which is unstable in blood, nor histamine which is devoid of dilator effect on the rabbit ear. Wybauw[120] noted an increase in both magnitude and duration of the antidromic dilator response in eserinized cat limbs. Similarly, eserinized Ringers perfusate of the limbs was found to lower the blood pressure of another cat. On the basis of these observations Wybauw proposed that the response was mediated through a cholinergic mechanism. An increase in gastric secretion reaching a maximum 8 to 10 minutes after stimulation of dorsal root fibers in the dog was observed by Ungar,[108] who proposed that the gastric effect was mediated by blood-borne histamine originating in the areas dilated by the antidromic stimulation. In subsequent publications Ungar and Parrot[109, 110] denied that this substance was histamine, and proposed that it resembled an adrenaline derivative in its chemical and pharmacologic properties. The material isolated from hindlimb venous effluent was in most tests (blood pressure, isolated tissues, and so on) indistinguishable from an enzymatic product of adrenaline, adrenoxine. The chemical nature of adrenoxine is unknown nor is it known whether this substance may release histamine. Kwiatkowski[72] in cats and Ibrahim and associates[67] in dogs have both utilized the same assay technique for histamine (method of Barsoum and Gaddum) and both claim to find an increase of blood histamine in venous effluent after antidromic stimulation. Ibrahim and co-workers established that 5 mg per kg of antistin, pyrabenzamine, and mepyramine were at least partially effective in abolishing antidromic vasodilatation. The ability of antihistamines to inhibit this type of dilatation has not been confirmed by other workers. Parrot and Lefebvre[86] claimed that the degree of dilatation from posterior root stimulation was unaffected by a dose of antergan which abolished an equivalent response resulting from the administration of histamine. Holton and Perry[66] were unable to alter antidromic dilatation in the rabbit ear by giving neoantergan. Pyribenzamine was reportedly ineffective against dorsal root dilatation in the cross-perfused hindlimb of the dog, but this same response was inhibited by a large dose of atropine (3 to 5 mg per kg), a dose level which, however, can produce ganglionic blockade[52] (see unpublished observations by Beck, below). Holton[65] succeeded in identifying adenosine triphosphate (ATP) in effluent from the perfused rabbit ear when the great auricular nerve was stimulated antidromically. This observation helps to substantiate the original suggestion of Holton and Holton[64] that ATP might well be the mediator of antidromic dilatation in the rabbit ear. It is curious that such a conflict of opinion should exist regarding antidromic dilatation but it

would seem that at least some of the discrepancy arises from a failure to carefully control the experiments and perhaps to a lesser extent the failure to consider species differences in the various investigations.

Histamine levels in the blood are reportedly increased during infusion of large quantities of epinephrine. Eichler and Barfuss[32] first reported such an elevation in cats during infusions of 9.5 to 28.5 μg per kg per min of epinephrine. The findings of Eichler and Barfuss have since been reported for man and for the dog.[28, 103, 104, 117] All of these authors believed that the release of histamine during the infusion of catecholamine represented a compensatory or "counter-regulatory" mechanism associated with the rise in blood pressure. Mongar and Whelan[85] were unable to confirm the results of Staub[103] in man. Using the same method for the estimation of histamine and the same quantity of epinephrine used by Staub, they were unable to find an increase in histamine titer in either arterial or venous blood. In favor of the work by Mongar and Whelan is that the average resting blood levels of histamine reported (10 μg per liter) are in the range that one would expect in man, whereas the resting levels reported by Staub went as high as 134 μg per liter with none below 16 μg per liter. This would seem to suggest that the substance Staub was measuring might not have been histamine. Whelan[119] has also reported that antihistamines are without effect on the increased forearm flow witnessed in man during intravenous epinephrine infusions.

An intriguing observation was made by Went and Varga[117] who discovered that chronic buffer nerve denervation preparations failed to show an elevation in blood histamine levels during epinephrine infusions. This is highly suggestive that the histamine release is reflex in nature, rather than a non-specific release from peripheral stores.

Histamine has been implicated as a mediator of post-exercise hyperemia. Anrep and Barsoum and associates[2, 4] stimulated the sciatic nerve of anesthetized dogs and observed an increase in the concentration of histamine in the venous blood emerging from the activated limb. A similar elevation of the histamine titer was noted upon direct stimulation of muscles whose innervation had been removed by prior section of the sciatic nerve. These results have never been confirmed, however. It is significant to note that Code and associates[26] were unable to confirm the results of Anrep and associates,[3] although they were able to show that cardiac muscle of the dog (heart-lung preparation) released histamine into the coronary sinus blood proportional to the work performed by the heart; *i.e.*, the stronger the heart, the greater the quantity of histamine released. Identical methods were used in each investigation. Whelan[119] studied the effect of several antihistamines on the increase in forearm blood flow after a period of exercise. In four subjects tripelennamine and antazoline did not alter the hyperemic response to exercise.

In summary, the information in the literature clearly shows that histamine is present in nervous tissue and is most concentrated in sympathetic post-ganglionic fibers. Although there appears to be no unequivocal evidence for the existence of a histaminergic vasodilator system in the literature, the distribution of histamine in the autonomic nerves is such that if a histaminergic system exists it would most likely be mediated over that portion of the autonomic system classically described as the sympathetic nervous system. This possibility is considered below.

SUMMARY OF STUDIES CARRIED OUT IN OUR LABORATORY RELATING TO ACTIVE REFLEX DILATATION AND ITS POSSIBLE MEDIATION BY HISTAMINE OR A CLOSELY RELATED SUBSTANCE*

The mechanism of reflex dilatation produced by the intravenous injection of epinephrine and norepinephrine has been under fairly intensive study in this laboratory for the past 5½ years. The study was stimulated by the observation that reflex dilatation induced by intravenous epinephrine could exceed the fall in blood pressure produced by ganglionic blockade. It was felt that the passive component of the reflex could not exceed the fall in perfusion pressure produced by chemical sympathectomy and therefore that a portion of the reflex must have been active in nature. Since a large portion of this work is not yet available in the literature it will be abstracted here to facilitate understanding of the discussion to be given before members of the symposium.

The basic method utilized in this investigation

* The work carried out in this laboratory and summarized in the symposium was supported by Grant H-3946, USPHS.

has been previously published.[10] Briefly, it consists of perfusing the innervated hindquarters with a sigmamotor pump. A retroperitoneal approach to the aorta is made in the flank area. Two pairs of dorsolumbar arteries and the inferior mesenteric artery are ligated to reduce collateral circulation to a minimum. The aorta is divided, a large polyethylene cannula is placed in the proximal aorta and blood led through a delay system to a sigmamotor pump. The blood is then pumped to the hindquarters through the descending aorta. The purpose of the delay, which consists of a glass coil immersed in a thermoregulated tank, is to retard the arrival of intravenously administered drug so that reflex action can become maximal in the perfused area before the intravenously injected drug arrives in the perfused area. With such a system, interpretation of the reflex response is not confounded by the local vascular actions of the injected drug.

A variety of experiments have been carried out utilizing this basic experimental design. It has been shown that reflex dilatation significantly exceeds the fall in perfusion pressure produced by hindquarter sympathectomy.[11] Sympathectomy in turn abolishes the reflex. Reflex dilatation produced by epinephrine or norepinephrine remains after abolition of tone by optimal doses of the norepinephrine antirelease agents demonstrating that active reflex dilatation can occur in the absence of sympathetic tone.[92] The beta-methyl derivative of TM 10 (SKF 6890A) is clearly better than TM 10, bretylium or quanethidine for the purpose of dissociating active and passive reflex dilatation. Additional evidence for active dilatation has been obtained by utilizing amine oxidase inhibitors to slow the degradation rate of norepinephrine. Under these circumstances active reflex dilatation can occur much more rapidly than passive dilatation. By use of the adrenergic antirelease agents and the amine oxidase inhibitors, it has been possible to demonstrate the presence of a large active component of reflex dilatation when considerable sympathetic tone exists, and therefore when the potential for passive reflex dilatation is large. Chronic pre-treatment with reserpine abolishes both reflex constriction and reflex dilatation, but acute treatment results in a rapid loss in reflex dilatation at a time when reflex constriction is actually enhanced. The mechanism whereby reserpine blocks active reflex dilatation is unknown. The blockade of passive reflex dilatation upon chronic treatment can be attributed to norepinephrine exhaustion.

Reflex dilatation is not affected by low doses of atropine sufficient to block the sympathetic cholinergic fibers. Larger doses of atropine are capable of reducing reflex dilatation but such doses also cause a parallel reduction in the constrictor response following pre-ganglionic stimulation of the sympathetic nerves indicating that ganglionic blockade occurs at high dose levels. Ganglion blocking agents block both reflex dilatation and reflex constriction showing that both the passive and active reflex systems are ganglionated. The beta adrenergic agent, dichloroisoproterenol, does not block reflex dilatation in doses which abolish an equivalent dilatation induced by the intra-arterial injection of isoproterenol. Dichloroisoproterenol does produce a considerable reduction in vascular tone in some animals which in turn reduces the absolute magnitude of the reflex but the reduction is not greater than that which occurs with comparable dilating doses of glyceryl trinitrate demonstrating that the reduction is non-specific in origin. The results obtained with dichloroisoproterenol show that the active reflex dilatation is not simply a phenomenon of epinephrine reversal resulting from a diminished neurogenic release of epinephrine nor can it be ascribed to the release of a substance with purely beta adrenergic stimulating activity.

Some of the antihistamines, particularly tripelennamine and D-chlorpheniramine, produce an essentially parallel reduction in reflex dilatation and in the dilatation produced by intra-arterial histamine.[11] Other antihistamines are generally not as effective but many produce a variable blockade. Dilatations produced by acetylcholine, isoproterenol, glyceryl trinitrate, bradykinin, substance P and other vascular dilators remain essentially unchanged following reflex and histamine blocking doses of tripelennamine. The blockade of reflex dilatation produced by the antihistamines does not appear to be a local anesthetic action since several local anesthetics, closely related to the antihistamines, but devoid of antihistaminic activity, fail to block reflex dilatation in larger doses. Cocaine, on the other hand, does markedly reduce reflex dilatation but its steroisomer,

tropacocaine, fails to block reflex dilatation in larger local anesthetic doses. It is interesting that cocaine has been reported to prevent the release of histamine whereas tropacocaine may even facilitate release.[68] The parallel between the two sets of observations is intriguing. A number of histamine releasers have been tried and many reduced reflex dilatation. Agents which reportedly potentiate the action of histamine on the isolated gut have been investigated for their potentiating effect on reflex dilatation. No regular potentiation of reflex dilatation nor of histamine induced dilatation was observed. In those experiments where potentiation occurred, the reactivity to glyceryl trinitrate was also potentiated suggesting a non-specific effect.

Several agents generally categorized under the heading of alpha adrenergic blocking agents were tested for their ability to block reflex dilatation. Dibenzyline usually produced considerable reduction in reflex dilatation. Since histamine induced dilatation was also markedly reduced by dibenzyline the loss of reflex activity cannot necessarily be ascribed to alpha adrenergic blockade. Sometimes blockade of histamine-induced dilatation even exceeded the degree of reflex blockade. Several of the ergot alkaloids blocked reflex dilatation almost completely in large doses. Lower doses, particularly of dihydroergocornine, produced a dramatic reduction in reflex dilatation without (a) producing a fall in perfusion pressure, (b) reducing the constrictor effect of sympathetic nerve stimulation, (c) decreasing the reflex constriction produced by asphyxia, afferent vagal stimulation or lowering of the blood pressure by a hypotensive agent, and (d) effecting a significant blockade of intra-arterial norepinephrine. It therefore seems unlikely that the reduction in reflex dilatation produced by dihydroergocorine is the result of adrenergic blockade, prevention of release of norepinephrine from the nerve endings, ganglionic blockade, central depression of the vasomotor center or blockade of afferent systems. Nor does dihydroergocornine block injected histamine. The specific mechanism of reflex blockade remains to be elucidated but it seems clear that the reflex dilatation cannot be arbitrarily classified as adenergic because these agents block the reflex in whole or in part.

Were reflex dilatation purely passive, alpha adrenergic blockade would remove effective sympathetic tone and consequently abolish passive reflex dilatation. If no active component were present maximal alpha adrenergic blockade, uncomplicated by extraneous drug actions, would therefore simulate the results obtained by sympathectomy, or ganglion blockade. If an active component were present, and the adrenergic blocker produced no effects other than alpha adrenergic blockade, the results would simulate those found with the norepinephrine antirelease agents.

The antiserotonins, 2-brom-D-lysergic acid diethylamide and 1-methyl methergine bimaleinate, were tested for their ability to modify reflex dilatation. Doses which were capable of blocking the vascular constrictor action of serotonin on the hindquarter circulation failed to block reflex dilatation. Such doses do not block the dilator action of serotonin. Since serotonin has been reported to release histamine the dilator action may well be due to release of histamine.[43] This seems the more probable in that we have been able to block the dilator response by administration of antihistamines. Much larger doses of the antiserotonin ergot derivatives are able to reduce or block reflex dilatation but this may be due to an action similar to the low dose effect reported with dihydroergocornine. Large doses of these antiserotonins may also block dilator action of serotonin thought to be due to histamine release and therefore could conceivably exert their effect through histamine antirelease action.

A study of the afferent systems involved in active reflex dilatation suggests that receptors capable of evoking it are located in the carotid sinus area and the distribution of the vagus as well as other areas. Application of increased pressure gradients to the sinus area by means of a second sigmamotor pump placed in the carotid circulation produces a reflex dilatation in the perfused hindquarters. The evoked reflex has both a transient and sustained component. The larger transient component usually lasts for less than a minute. The sustained component does not seem to accomodate even when the increased gradient is maintained for several minutes. The transient component is believed to be active since it can be obtained after the abolition of sympathetic constrictor tone with the antirelease agent SKF 6890A and can be blocked by antihistamine. The sustained component is believed to represent passive reflex dilatation because it is not blocked

by antihistamine but disappears after treatment with the antirelease agent. Stimulation of central sinus or vagus nerves after sinusectomy and vagotomy results in reflex dilatation. Prolonged stimulation of the sinus nerves results in both the transient and sustained types of reflex dilatation whereas stimulation of the central vagus usually results in only the transient component.

Administration of epinephrine during inhibition of sympathetic tone by high pressure perfusion of the sinuses still results in a transient component of dilatation. After vagotomy and effective sinusectomy (verified by the fact that increased pressure no longer evokes a reflex) epinephrine can evoke reflex dilatation but is often less pronounced than the reflex dilatation occurring before denervation of the buffer nerves. Before sinusectomy and vagotomy norepinephrine is a more potent reflexogenic agent than epinephrine, but after these procedures epinephrine is more potent than norepinephrine.

Veratrine and veratridine produce an active reflex dilatation in the perfused hindquarters along with a systemic hypotension. The reflex dilatation and the histamine induced dilatation are blocked essentially in parallel by antihistamine. Veratrine induced reflex dilatation is abolished by combined vagotomy and sinusectomy but usually not by vagotomy alone.

Stimulation of the intact lumbar sympathetics at appropriate parameters (generally low voltage, short impulse duration) can produce rather prominent dilatation in the perfused area which can be blocked by antihistamine. Increasing the voltage and impulse duration following histaminic blockade produces a vascular constriction demonstrating an absence of local anesthesia. After SKF 6890A stimulation of the sympathetics results in a considerable dilatation in the perfused area partially amenable to blockade by atropine and partially by antihistamine.[92] After chronic pre-treatment with reserpine or SU 4029 electrical stimulation produces a dilatation which can be blocked by atropine but not by antihistamines. Sometimes, especially after treatment with atropine, a minor dilatation, which at present we have been unable to block, occurs slowly and remains for sometime after the end of the stimulus period. After chronic treatment with reserpine and SU 4029, reflex dilatation and reflex constriction are usually abolished, *in toto*, an observation which is compatible with the failure to block any component of the electrically induced dilatation by antihistamines following these agents.

Attempts to recover histamine from the venous effluent following elicitation of the reflex have not been successful. It appears from recovery experiments, following intra-arterial infusion of histamine, that the histamine which diffuses out of the blood vessel fails to re-enter the vascular system. These results are suggestive of a rapid termination or binding of histamine by the tissues. No evidence for a reduction in norepinephrine content in the venous effluent was obtained either during the reflex or following local hindquarter sympathectomy. Intense lumbar sympathetic stimulation, estimated to produce a 16-fold increase in norepinephrine secretion (based on an assumed log-dose response relationship which occurs with intra-arterial infusion) increased the venous concentration of norepinephrine to barely detectable levels. The results of the assays for neurohumoral substances therefore support neither the thesis of active nor passive reflex dilatation.

Tracheal occlusion produces an abrupt reflex constriction in the perfused area. When the trachea is de-occluded a sharp dilatation occurs. This dilatation is active in nature and reflex in origin. It is abolished by sympathectomy and adequate ganglionic blockade. It may remain in large part after adequate dihydroergocornine to abolish the active component of reflex dilatation produced by intravenous epinephrine or norepinephrine. Tracheal de-occlusion dilatation is not blocked to any extent by antihistamines unless tracheal occlusion produces an associated rise in blood pressure. In this case the tracheal occlusion reflex is evoked in conjunction with the catecholamine-induced reflex resulting from adrenal release. It is this latter component which is blocked by antihistamines when tracheal occlusion produces a rise in blood pressure. The tracheal de-occlusion reflex may at times be partially blocked by relatively large doses of atropine. As indicated above, Dr. Aviado[82] has recently reported active reflex dilatation following hypoxia in animals treated with bretylium and reported that the reflex is partially blocked by atropine. In view of our observation that doses of atropine from 0.5 to 3.0 mg per kg can decrease the constrictor response produced by lumbar sympathetic stimulation we no longer feel secure in necessarily

attributing the blocking effect of atropine on this reflex to blockade of a cholinergic vasodilator component. Nevertheless, it seems apparent that the greater portion of this type of active reflex dilatation is mediated by a different substance than the reflex dilatation produced by the intravenous injection of epinephrine and norepinephrine.

THE NATURE OF RELEASE OF MEDIATORS FROM NERVE ENDINGS

A curious and interesting observation arose out of the investigation dealing with the norepinephrine antirelease agents. The original aim was simply to demonstrate that active reflex dilatation could be preserved at a time when norepinephrine secretion had been blocked. It was fortunate, but purely fortuitous, that both the first agent and the employed dose were optimal for this demonstration, otherwise the investigation may never have been completed. It was soon discovered that other antirelease agents did not always preserve reflex dilatation at doses which were required to block norepinephrine release. In fact, at times reflex dilatation was blocked out of proportion to the norepinephrine antirelease effect as demonstrated by various procedures designed to evoke reflex constriction. This observation prompted an investigation into the higher dose effects with beta TM 10. Progressive increments in dose caused, at first, a fairly rapid loss in reflex constrictor ability with only moderate reduction in reflex dilatation, indicating that the lower doses had effectively removed sympathetic tone. At these doses maximal stimulation of the lumbar sympathetics produced only a fall in perfusion pressure as opposed to a large rise previous to beta TM 10. As the dose was further increased no additional reduction in perfusion pressure occurred, but a progressive diminution in the remaining reflex dilatation could be obtained.

These observations suggested that the norepinephrine antirelease agents were capable of preventing release not only of norepinephrine but also, in appropriate doses, of the active dilator which mediated the reflex dilatation induced by norepinephrine injection. It had been shown previously by Exley[42] that the sympathetic nerves still conducted impulses following the antirelease agents and we had observed that agents like *d*-amphetamine could still produce a large pressor response at a time when sympathetic stimulation failed to produce constriction following the antirelease agents. The fact that these agents had some structural resemblance to other quaternary compounds which blocked acetylcholine at the ganglia and neuromuscular junction, coupled with the observations above, raised the theoretical question of whether these agents might be blocking an acetylcholine action. The working hypothesis was put forward that norepinephrine and the active dilator mediating reflex dilatation might be contained in independent packets at different nerve endings or at neuroeffector sites, that a nerve impulse passing down the sympathetics would release a quantum of acetylcholine which in turn would produce a temporary depolarization of the packet permitting the secretion of an aliquot of norepinephrine or the active dilator depending on which sympathetic nerves were physiologically activated. The packet would then repolarize as the secreted acetylcholine was hydrolyzed and secretion of the mediator would stop. It was postulated that the antirelease agents might prevent the depolarization of the packet by acetylcholine and thus effect an antirelease action. The hypothesis was tested by setting up a hindquarter perfusion preparation similar to that referred to above.

Hemicholinium, an agent which prevents synthesis of acetylcholine, was given. The animal was placed on artificial respiration since respiration will cease after this compound when the physiologic stores of acetylcholine are depleted. The lumbar sympathetics were repetitively stimulated at short intervals and intravenous norepinephrine was given intermittently.

These maneuvers were carried out with idea of depleting existing depots of acetylcholine at the theoretical packet. In 2 of 7 animals sympathetic tone and reflex dilatation disappeared completely within 2 to 4 hours. In one of these dogs the intravenous administration of choline (which has been previously demonstrated to antagonize hemicholinium) restored both sympathetic tone and reflex dilatation. In the other animal a minor return of sympathetic tone only was obtained.

These results were compatible with the hypothesis put forth above that norepinephrine and the active dilator are released through the intermediate action of acetylcholine secretion by the sympathetic nerves. The soundness of the inference, however, was clearly based on whether

pre-ganglionic fibers or post-ganglionic fibers were being stimulated. If pre-ganglionic fibers were stimulated, exhaustion of acetylcholine could occur at the ganglion and thus lead to a misinterpretation of our data. Before increasing our series the counter experiment of stimulating the lumbar sympathetics and examining the degree of blockade with ganglion blocking agents was therefore carried out. It was found that large doses of hexamethonium, tetraethylammonium, azomethonium, pempidine and ostensin reduced the effects of low lumbar and even upper sacral stimulation from 60 to 100 per cent. From the results obtained with the ganglion blocking agents it becomes clear that the hypothesis put forward above, that acetylcholine is involved in the release of norepinephrine and the dilator substance, must remain hypothetical until the alternative possibility that the hemicholinium blockade occurs at another site is ruled out. If the site of stimulation in our experiments were post-ganglionic it would require a change in views on the mechanism of action of the ganglion blocking agents.

It should be pointed out that Burn and Rand[20] have also proposed that norepinephrine release is brought about through the intermediary action of acetylcholine. These authors argue that acetylcholine is involved in the release of norepinephrine for a number of reasons, the most important of which are as follows: (1) Stimulation of sympathetic nerves to various areas before exhaustion by the releasing agent, reserpine, produced a vasoconstriction; after exhaustion stimulation of the same nerves produced a dilatation, potentiated by physostigmine, and amenable to block by atropine. (2) Acetylcholine and nicotine can produce a constriction in the rabbit ear before, but not after, degeneration of nerve fibers. (3) Stimulation of the lumbar sympathetic chain for 3 seconds produced a decrease in arterial resistance to the hindleg of the dog but stimulation for 30 seconds produced a rise in resistance. (4) Stimulation of the sympathetic fibers to the rabbit atria normally causes acceleration in rate. (5) Koelle has observed cholinesterase in sympathetic nerves which are generally considered to be predominantly adrenergic (for more detailed discussion and references see original article by Burn and Rand[20]).

Unquestionably the above observations are consonant with the hypothesis put forward by Burn and Rand, but it is worthwhile to review these considerations from an alternative standpoint. The autonomic nerves generally classified as sympathetic may contain, as anatomically distinct entities, both cholinergic and adrenergic fibers as well as fibers mediated by other substances. Strong electrical stimulation of these compound nerves may produce a predominant release of norepinephrine which masks the simultaneous release of cholinergic dilator material (this could also apply if other dilators in addition to acetylcholine were released). Depletion of norepinephrine depots with reserpine or prevention of norepinephrine release by antirelease agents would now permit an unmasking of the effect of stimulation of dilator fibers previously masked by the greater secretion of norepinephrine from adrenergic fibers. This "masking" concept was originally proposed by Dale in 1906[29] and it would still seem a tenable viewpoint even today. The same mixed nerve interpretation could explain the effect of stimulation of sympathetic nerves to the rabbit atria.

If a sympathetic cholinergic vasodilator system did not exist as a distinct entity it would be virtually impossible to explain the observations of the Scandinavian workers (see section on Cholinergic Mediation of Active Dilatation) that central stimulation produced a profound vascular dilatation in the hindlimb of the cat, transmitted over the sympathetics, and blocked by atropine. Clearly this secretion of acetylcholine cannot involve secretion of norepinephrine; otherwise a constriction would occur since this system is operative in non-drug-treated animals. The lesser dilatation observed in other sympathetically innervated areas upon electrical stimulation of the sympathetics following norepinephrine exhaustion by reserpine might simply reflect a less dense distribution of dilator fibers to those areas. Similarly, the observation that cholinesterase is present in sympathetic nerves may be taken to indicate that cholinergic fibers are present in such systems or that acetylcholine is involved in the normal function of many types of nerves. The observations of Koelle and Koelle[70] that only a small proportion of the sympathetic ganglion cells stain densely is strongly suggestive that at least two physiologically discrete types of cells exist in the sympathetic ganglia. The constriction produced by acetylcholine and nicotine in the rabbit ear

before, but not after degeneration, is a strong argument for the above hypothesis; but even in this instance the ability of an agent to promote release of norepinephrine does not necessarily implicate that substance as the physiologically active agent, for many other substances can also discharge catecholamine.

The argument regarding the immediate and prolonged effects of lumbar sympathetic stimulation is not a telling one. Similar observations quoted by Burn have also been observed in this laboratory. But we have also found it is possible to obtain only dilator effects at the same electrode placement, irrespective of the duration of stimulation, if appropriate parameters of stimulation are chosen. Such dilator effects soon subside, usually within 30 seconds, even though no secondary constrictor phase occurs. Discontinuing the stimulation for a short period and then repeating the stimulation permits the reappearance of the dilatation. By contrast, marked constriction in the innervated area produced by strong stimulation of the lumbar sympathetics can be maintained for long periods. If the initial dilatation resulted from the release of acetylcholine whose function was to promote the release of norepinephrine, then such release of acetylcholine should continue as long as the stimulation is maintained. The transient nature of the dilatation following electrical stimulation cannot be ascribed to a masking effect of the dilatation by a delayed release of constrictor substance because continuous electrical stimulation, even at high voltages, following norepinephrine exhaustion with reserpine or following prevention of norepinephrine release by the antirelease agents, produces a marked dilatation which also soon subsides. This is similar to the effect in the untreated animal stimulated at appropriate parameters.

Chang and Rand[24] have very recently published a paper in support of their hypothesis using hemicholinium. They report that treatment with hemicholinium abolished the contraction of the vas deferens produced by hypogastric stimulation, the inhibition of uterine contraction by electrical stimulation of the hypogastric nerve, inhibition of the colon by lumbar sympathetic stimulation, and constriction of the rabbit ear by stimulation of the superior cervical ganglion. They reported in many instances partial reversal of the depressed responses could be achieved by administration of choline. These observations are in strong support of their thesis providing ganglionic blockade and effects on nerve conduction can be ruled out. Although the authors feel that they are stimulating at post-ganglionic sites in many instances, they apparently have not tested for the presence of ganglia by administration of a test dose of a large amount of ganglion blocking agent. In view of our own findings that the ganglion blocking agents usually abolished most of the dilatation and constriction produced by lumbar sympathetic stimulation in our dog-hindquarter preparations, it would seem wise to utilize a test agent to confirm that the systems used by Chang and Rand in the above investigation were post-ganglionic. On the other hand it may be that such large doses of ganglion blocking agent block release from post-ganglionics. Caution must also be excercised in interpretations of experiments in which nerve stimulation is carried on for several hours, for in some instances the response becomes depressed or disappears altogether without administration of drug. Unless the response is reversible by choline, the result should likely be discarded. A further thought to be borne in mind is whether transmission fails in the nerve rather than at its ending. The observation that, after hemicholinium, phrenic nerve activity still occurs at a time when neuromuscular transmission is lost, does not necessarily permit the inference that the nerve is the least susceptible link in the chain of other systems.[83]

The concept expressed by Burn and Rand[20] that acetylcholine serves as an intermediate in the neurogenic release of norepinephrine is most certainly intriguing for it would give a unifying concept of neurohumoral release and it would also represent yet another demonstration of the already magnificent receptor specificity to pharmacologic agents capable of blocking processes excited by acetylcholine. At the moment, however, it would seem that the results should be accepted with some measure of reserve until all possible objections have been removed.

Although our own hypothesis is generally in accord with the view of Burn and Rand it appears to differ fundamentally in that we believe the sympathetics contain some discrete cholinergic fibers which release acetylcholine at their post-ganglionic endings as the neuro-effector

substance, *per se*, and that this acetylcholine is capable of producing marked dilatation without norepinephrine secretion. Anatomically different fibers secrete norepinephrine through the depolarizing action of acetylcholine on packets either within the nerve or in exquisite proximity to this "adrenergic" ending. Local destruction of this latter acetylcholine would normally occur rapidly enough to prevent its exerting a vascular action. We also envisage other dilator fibers which liberate histamine or a substance with similar pharmacologic properties. This dilator substance is released through a mechanism similar to the release of norepinephrine and is responsible for the active component of reflex dilatation brought about by injection of epinephrine, norepinephrine and the stimulation of various afferent systems. There is also the problem of explaining the active reflex dilatation produced by asphyxia since it is also transmitted over the sympathetics and appears to be different at least in part from the system of Folkow and Uvnäs, and that mediating active dilatation induced by intravenous norepinephrine and epinephrine.

Thus there is at least some evidence for the existence of at least four functionally independent systems. It is difficult to envisage how these systems can act more or less independently unless they are mediated over separate pathways. The release of different mediators from different "nerve endings" by the same agent would not seem incompatible with the hypothesis that each mediator is released from anatomically different fibers which are conducted to the periphery in the same compound nerve.

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