Peripheral Sympathectomy and Adrenal Medullectomy Do Not Alter Cerebrospinal Fluid Norepinephrine

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Despite a blood–brain barrier for norepinephrine, the concentration of norepinephrine in plasma and cerebrospinal fluid has been observed to be similar. This relationship between plasma and cerebrospinal fluid norepinephrine levels suggests that peripheral sympathetic neurons innervating blood vessels to brain and spinal cord may contribute significantly to cerebrospinal fluid norepinephrine levels, and questions the validity of cerebrospinal fluid norepinephrine as an index of central nervous system noradrenergic activity. We demonstrate that extensive destruction of the peripheral sympathetic nervous system and the adrenal medulla has no effect on rat cerebrospinal fluid norepinephrine. It is therefore unlikely that peripheral sources of norepinephrine contribute significantly to cerebrospinal fluid norepinephrine levels.

INTRODUCTION

Cerebrospinal fluid (CSF) norepinephrine (NE) concentration has been used as an index of central nervous system (CNS) noradrenergic activity in studies of such diseases as hypertension[19], schizophrenia[20], depression[31], amyotrophic lateral sclerosis[39], and Alzheimer's disease[32]. The assumption that CSF NE is a valid measure of brain noradrenergic activity is based upon several factors. Noradrenergic cell bodies in the locus coeruleus and rostrally projecting subcoeruleus nuclei provide a particularly rich noradrenergic innervation to brain areas in close proximity to the CSF and caudally projecting noradrenergic nuclei also innervate the spinal cord[25,35]. Also, norepinephrine injected into the CSF is taken up by catecholaminergic neurons of central rather than peripheral origin[8]. In addition, an effective blood–brain barrier for NE has been well documented[41]. However, despite this blood–brain barrier for NE,

there is a close correlation between NE in CSF and plasma when the two compartments are sampled simultaneously[32,40]. Because plasma NE reflects peripheral sympathetic nervous system (SNS) activity[17], the close relationship between CSF and plasma NE suggests that either NE release in both compartments is jointly regulated, or that the peripheral SNS somehow contributes significantly to CSF NE levels as well as to plasma NE levels. If the peripheral SNS does contribute significantly to CSF NE, the validity of CSF NE as an index of CNS noradrenergic activity is suspect.

We investigated the contribution of the peripheral SNS to CSF NE levels by measuring CSF NE following chemical ablation of the SNS and adrenal medullectomy. Chronic administration of guanethidine to neonatal rats produces peripheral sympathectomy but spares central noradrenergic neurons and the adrenal medulla[15]. In this study, we measured CSF and plasma NE concentrations in guanethidine-sym-

pathectomized rats, guanethidine-sympathectomized/adrenal medullectomized rats, and sham-injected/sham-operated control rats. Measurements of plasma NE following decapitation and tissue NE content in hypothalamus, cerebral cortex, heart, and adrenals provided indices of the effectiveness of the guanethidine sympathectomy and adrenal medullectomy procedures. In addition, we measured CSF and plasma epinephrine (E) concentrations and adrenal E content in the 3 groups.

MATERIALS AND METHODS

Chemical sympathectomy was performed in 40 male Sprague–Dawley rats beginning at one week of age by administering 5 daily subcutaneous injections of 50 mg/kg guanethidine monosulfate (Ciba Geigy) dissolved in 10 μl/g body weight of normal saline per week for 3 weeks[15]. Sixteen littermates received an identical course of normal saline. All rats were weaned at 4 weeks of age and housed 2 to a cage with free access to standard laboratory rat chow and tap water.

At age 7 weeks, guanethidine-treated rats underwent either bilateral enucleation of the adrenal gland[38] or sham surgery. All control rats underwent sham surgery. The adrenal cortex but not the adrenal medulla regenerates after adrenal enucleation, thus producing a functionally adrenal medullectomized animal[13,34]. Adrenal medullectomy took place under ether anesthesia. A single midline dorsal skin incision was made and the lateral abdominal wall parted on each side to expose the adrenals. Each adrenal was carefully lifted, a small incision made in the capsule with microscissors, and the body of the gland extruded with blunt forceps. The skin incision was closed with wound clips. The sham operation consisted of the above procedure through exposure of the adrenal capsule followed by closure of the skin incision. Operated rats received 0.9% saline in place of tap water to drink for the first 7 days following surgery and then a choice of saline or tap water for an additional 7 days[38]. Saline was provided to compensate for salt and water losses during the period of corticosteroid deficiency. Sham-operated rats received only tap water to drink.

At the end of the study, control rats weighed significantly more (394 ± 9 g) than either sympathecto-

mized (330 ± 9 g) or sympathectomized/adrenal medullectomized (327 ± 10 g) rats ($F_{2,38}$ = 16.66, P < 0.001). Sympathectomized rats developed marked ptosis which persisted for the duration of the study. Fifteen sympathectomized rat pups but no control group rat pups died at some point during the study.

At 12 weeks of age, CSF and tail vein samples were collected under ketamine anesthesia (100 mg/kg i.p.). Ketamine does not affect baseline plasma catecholamine concentrations in primates[4,16]. After nicking the tail vein, 200 μl of blood were collected into iced tubes containing heparin and reduced glutathione. Immediately following tail vein sampling, approximately 150 μl of CSF were collected from the cisterna magna[36]. The rat was mounted on a stereotaxic device modified to position the head at a 90° angle to the body. The location of the cisterna magna was estimated by setting the dorsal–ventral drive of the stereotaxic 6.8 mm below the top of the skull. A 30-gauge needle attached to PE 10 polyethylene tubing was advanced through a small skin incision into the cisterna and the CSF was allowed to flow by gravity into iced tubes containing reduced glutathione.

After fully awakening from anesthesia (15–20 min), the rats were decapitated and trunk blood collected into tubes containing iced heparin and reduced glutathione. Decapitation of unanesthetized rats produces a 10-fold increase in trunk blood NE and E levels[30]. The brain was rapidly dissected on an iced glass plate and the hypothalamus and cerebral cortex blotted dry, weighed and frozen on dry ice. Hearts and adrenal glands also were rapidly removed, rinsed with iced saline, blotted dry, weighed and frozen on dry ice. All CSF, plasma and tissue samples were stored at –70 °C.

CSF and plasma NE and E levels were determined by a sensitive radioenzymatic method[5,29]. Tissue NE and E levels were determined by reverse-phase high-performance liquid chromatography (HPLC) with electrochemical detection.

Adrenal (40 ± 2 mg) and hypothalamic (59 ± 3 mg) samples were homogenized by sonication (Branson Cell Disrupter 200) in 1.0 ml cold 0.1 N $HClO_4$. Cortex samples (0.51 ± 0.02 g) were similarly homogenized in 4.0 ml perchloric acid. Heart samples (1.10 ± 0.03 g) were Polytron (Brinkmann) homogenized in 15 ml $HClO_4$ prior to sonication. Samples

were centrifuged at 25,000 g for 20 min at 4 °C and the supernatant was removed and stored at -70 °C; 10 μg of 3,4-dihydroxybenzylamine (DHBA) was added as an internal standard to 0.80 ml of the supernatant from control and sympathectomized rat adrenal extracts, and 200 ng was added to the same volume of medullectomized adrenal extracts. Acid-prepared alumina (200 mg) and 0.5 M Tris/2% EDTA, pH 8.6, buffer (1.5 ml) were added to each thawed adrenal extract. Each sample was mixed for 15 min, centrifuged, and the supernatant discarded. The alumina was washed 3 times with 2.0 ml distilled water and the catecholamines eluted from the alumina with 1.0 ml 0.1 N $HClO_4$. Medullectomized adrenal extracts were used undiluted for HPLC, and control and sympathectomized rat adrenal extracts were diluted 1:100 in 0.1 N $HClO_4$. Extraction of other tissues was identical except for quantities of standard and reagents. For hypothalamus, 100 ng DHBA, 50 mg alumina, and 1.0 ml Tris buffer were added to 0.85 ml of the homogenization supernatant. Catecholamines were eluted with 0.4 ml 0.1 N $HClO_4$ and diluted 1:3 for HPLC. For cortex, 200 ng DHBA, 50 mg alumina, and 4.0 ml Tris buffer were added to the supernatant. Catecholamines were eluted with 0.4 ml 0.1 N $HClO_4$ and diluted 1:40. For heart, 200 ng DHBA, 200 mg alumina, and 15 ml Tris buffer were added to the total supernatant. Catecholamines were eluted with 0.8 ml 0.1 N $HClO_4$; approximately half of the samples were diluted 1:4 for HPLC and the rest were undiluted. HPLC injection volume for all extracts was 10 μl.

A Varian 5060 high-pressure liquid chromatograph with Varian MCH-5 30 cm × 4 mm reverse-phase column was used for catecholamine separation. The mobile phase consisted of 0.1 M sodium acetate, 0.02 M citric acid, 10 mg/l 1-octanesulfonic acid (sodium salt monohydrate), 50 mg/l sodium EDTA and 10% methanol. The flow rate was 1.0 ml/min. Detection was accomplished using a TL-5 glass carbon electrode and LC-17 flow cell (Bioanalytical Systems) and amperometric detector (LC-4A, Bioanalytical Systems) with an applied potential of +0.65 V vs Ag/AgCl.

A 0.1 N $HClO_4$ solution containing 20 ng/ml each of norepinephrine bitartrate, epinephrine, dopamine, and DHBA hydrobromide was injected on to the HPLC column in variable volumes (10–50 μl) to

yield standards of 0.2–1.0 ng. Peak heights were measured on the chromatographic chart recording and a linear standard curve prepared for each standard. Extraction efficiency was calculated for each sample by determining the concentration of DHBA recovered and correcting the measured values for norepinephrine and epinephrine accordingly[9,24]. Extraction efficiencies for each tissue were: adrenal, 77.6 ± 0.8%; hypothalamus, 87.7 ± 1.5%; cortex, 82.1 ± 0.6%; heart, 54.1 ± 1.0%.

RESULTS

NE levels in CSF, tail vein plasma and post-decapitation plasma are shown in Fig. 1. In control animals, tail vein NE levels were similar to CSF NE levels. Tail vein plasma NE levels were reduced to 33% of control levels in the sympathectomized rats and 39% of control levels in sympathectomized/adrenal medullectomized rats ($F_{2,38} = 25.08$, $P < 0.001$). However, despite the marked reduction of tail vein plasma NE in sympathectomized rats, CSF NE did not differ between sympathectomized rats and controls ($F_{2,38} = 0.74$, $P = $ n.s.).

The effectiveness of sympathectomy and adrenal medullectomy was demonstrated by postdecapitation NE levels, which were reduced to 46% of control values in sympathectomized rats and 17% of control values in sympathectomized/adrenal medullectomized rats ($F_{2,38} = 37.77$, $P < 0.001$). The effectiveness of these procedures was further supported by the tissue catecholamine levels (Tables I and II). Heart NE content was markedly reduced to 2% of control content in sympathectomized rats and 4% of control content in sympathectomized/adrenal medullectomized rats ($F_{2,38} = 523.02$, $P < 0.001$). Adrenal NE and E content in sympathectomized/adrenal medullectomized rats were reduced to 2% of control content ($F_{2,38} = 147.26$, $P < 0.001$, and $F_{2,38} = 207.20$, $P < 0.001$, respectively). Guanethidine sympathectomy spared CNS noradrenergic neurons as demonstrated by the absence of differences in hypothalamic NE content ($F_{2,38} = 2.33$, $P = $ n.s.) and cerebral cortex NE content ($F_{2,38} = 0.13$, $P = $ n.s.) among groups. Guanethidine sympathectomy also spared the adrenal medulla; in fact, adrenal NE was significantly higher in sympathectomized rats than in controls.
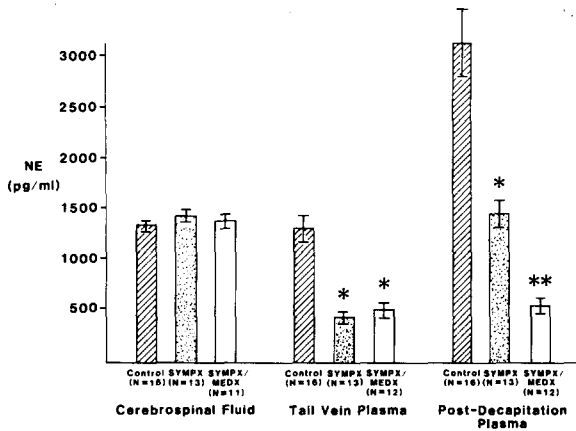
Fig. 1. Norepinephrine (NE) levels (pg/ml) in cerebrospinal fluid, tail vein plasma, and postdecapitation plasma in guanethidine-sympathectomized rats (Symp X), guanethidine sympathectomized and adrenal medullectomized rats (Symp X/Med X), and control rats. Values are means ± S.E.M. * $P < 0.001$ compared to control group; ** $P < 0.001$ compared to Symp X and control groups; analysis of variance followed by Scheffe's test.

E levels in CSF, tail vein plasma and postdecapitation plasma are presented in Table III. Tail vein plasma E levels are markedly higher than CSF E levels in all groups. Although tail vein plasma E tended to be lower in the sympathectomized/adrenal medullectomized rats, this difference was not significant. Postdecapitation plasma E was markedly lower in the sympathectomized/adrenal medullectomized rats than in the other two groups ($F_{2,38}$ = 23.69, $P < 0.001$).

DISCUSSION

This study demonstrates that extensive destruction

TABLE I

Norepinephrine levels (ng/g tissue) in hypothalamus, cortex and heart

Values are means ± S.E.M. Symp X, guanethidine sympathectomized rats; symp X/Med X, guanethidine sympathectomized/adrenal medullectomized rats.

Group	Hypo-thalamus	Cortex	Heart
Control (n = 16)	1473 ± 63	232 ± 7	917 ± 33
Symp X (n = 13)	1456 ± 70	238 ± 9	21 ± 6*
Symp X/Med X (n = 12)	1288 ± 66	238 ± 10	34 ± 12*

* $P < 0.001$ compared to control group (analysis of variance followed by Scheffe's test).

TABLE II

Norepinephrine (NE) (ng/g tissue) and epinephrine (E) (ng/g tissue) in whole adrenal gland

Values are means ± S.E.M. Symp X, guanethidine sympathectomized rats; Symp X/Med X, guanethidine sympathectomized/adrenal medullectomized rats.

Group	NE	E
Control (n = 16)	210 ± 1	800 ± 33
Symp X (n = 13)	279 ± 15*	870 ± 40
Symp X/Med X (n = 12)	4 ± 2**	19 ± 10**

* $P < 0.001$ compared to control group
** $P < 0.001$ compared to control and Symp X groups. Statistical comparison is by analysis of variance followed by Scheffe's test.

of the peripheral SNS does not affect CSF or brain NE levels in rats. Evidence for extensive destruction of peripheral SNS neurons includes the marked reduction in cardiac NE content. In addition, plasma NE levels, which appear to reflect NE spillover from sympathetic neurons innervating blood vessels[17], were considerably lower in sympathectomized animals both in tail vein samples and after the stress of decapitation. Despite this evidence of extensive destruction of peripheral SNS neurons, sympathectomy had no effect on brain NE content and CSF NE levels were unaltered.

Our results support previous findings that guanethidine sympathectomy produces an extensive and long-lasting sympathectomy, while sparing central noradrenergic neurons and the adrenal medulla. Heart NE levels were consistent with previous studies which reported profound reductions in cardiac

TABLE III

Epinephrine (pg/ml) in cerebrospinal fluid and plasma

Values are means ± S.E.M. Symp X, guanethidine sympathectomized rats; Symp X/Med X, guanethidine sympathectomized/adrenal medullectomized rats.

Group	CSF	Tail vein plasma	Postdecapitation plasma
Control (n = 16)	70 ± 5	924 ± 155	7841 ± 1007
Symp X (n = 13)	92 ± 10	658 ± 113	5554 ± 748
Symp X/Med X (n = 12)	71 ± 11	548 ± 95	166 ± 46*

* $P < 0.001$ compared to control and Symp X groups (analysis of variance followed by Scheffe's test).

NE content to between 2% and 22% of control values[1,2,7,10,15,21] following various protocols for guanethidine treatment in neonatal and adult rats. Our finding of unchanged hypothalamic and cerebral cortical NE content in guanethidine-treated rats is consistent with the reports of Johnson et al.[14,15] who found no differences in NE content in whole brain, spinal cord, or cerebellum in guanethidine-treated neonatal and adult rats as compared to controls.

A possible mechanism by which the peripheral SNS could contribute to CSF NE is via SNS neurons which innervate blood vessels to brain and spinal cord areas adjacent to the cerebral ventricles and the subarachnoid space. A prominent peripheral SNS innervation which degenerates following bilateral superior cervical ganglionectomy has been described in the choroid plexus and pial blood vessels of the rat and the pial vasculature of the cat[6,11,22,26]. Fibers with typical catecholamine fluorescence closely associated with blood vessels in the subarachnoid space and pia have been described below the level of spinal cord transection in the chronic spinal rat and dog[23]. Because chronic administration of guanethidine to neonatal rats produces peripheral sympathectomy with essentially complete destruction of the superior cervical ganglion as documented by electron microscopy[3,12], it is unlikely that SNS neurons innervating CNS blood vessels contributed to CSF NE in our sympathectomized rats.

Our finding of increased NE content in the adrenal glands of guanethidine-sympathectomized rats is consistent with that of Kvetnansky et al.[18], who found adrenal NE content elevated to 170% of control levels in their guanethidine-treated adult rats. Presumably, the increase of NE content represents a compensatory response of the adrenal medulla to destruction of sympathetic neurons. Their finding of profoundly reduced adrenal NE levels in guanethidine-sympathectomized/adrenal medullectomized rats was also similar to our results.

Tail vein plasma NE levels in our control rats were higher than those reported in unstressed, unanesthetized rats (from which blood samples were collected via an indwelling cannula), but were approximately midway between the levels reported in cannula blood from gently handled rats and rats subjected to immobilization stress[30]. The high tail vein NE levels in our control rats occurred despite the use of ketamine anesthesia, which has been reported to abolish the catecholamine response to acute cold exposure in monkeys[16]. It appears that the amount of ketamine anesthesia used in this study did not completely abolish the NE response to immobilization and tail incision involved in our blood sampling procedure.

Decapitation produced increased NE levels in control rats consistent with previously reported data in unanesthetized normal rats[30]. Decapitation in guanethidine-sympathectomized and guanethidine-sympathectomized/adrenal medullectomized rats produced plasma NE levels considerably lower than those in control rats, but higher than previously reported during immobilization stress in guanethidine-sympathectomized and guanethidine-sympathectomized/adrenal medullectomized rats[18]. However, in normal unanesthetized rats, decapitation produces plasma NE levels approximately 3 times higher than immobilization.

Guanethidine treatment produced a significant degree of mortality and growth deficit. Our guanethidine-treated rats exhibited an increased mortality with 15 of 40 treated rats dying during the study period compared to zero of 16 control rats. Treated rats also showed a persistent and significant growth deficit, amounting to 17% at 12 weeks of age. Johnson et al.[15], using the identical treatment protocol, reported an excess mortality of 9% in guanethidine-treated rats as compared to 13% mortality in our treated rats not attributable to anesthesia or surgical procedures. They also reported a transient growth reduction of 15% by the third week of treatment which disappeared by 10–11 weeks of age. In an earlier study involving young adult rats[14] and using a somewhat different treatment protocol (40 mg/kg 5 days a week for 5 weeks), they reported a growth deficit in treated rats which was significant by 3 weeks of treatment and which persisted for at least 6 months.

The large gradient between plasma E and CSF E supports the existence of a blood–brain barrier for catecholamines. This barrier has previously been demonstrated by radiolabeled catecholamine infusion studies in cats and primates[27,33,37], and by demonstration of a large gradient between CSF and plasma NE in a patient with pheochromocytoma, a NE-producing tumor of the adrenal medulla[41]. This pheochromocytoma patient had an extremely high plasma NE concentration of 9680 pg/ml, but a simul-

taneously normal CSF NE concentration of 200 pg/ml.

The plasma E levels in the tail vein samples from sympathectomized/adrenal medullectomized rats suggest secretion of E by extra-adrenal chromaffin tissue[28] in response to the tail vein nicking procedure. The decrease in plasma E levels after decapitation may result from an inability to maintain stress-elevated plasma levels or to secrete enough E to elevate plasma concentrations in response to a second stress within a short period of time.

These results demonstrate that extensive destruction of peripheral SNS noradrenergic neurons and the adrenal medulla has no effect on CSF or brain levels of NE. It is highly unlikely that peripheral sources of NE contribute significantly to CSF NE.

These results, therefore, strengthen the validity of CSF NE levels as a measure of CNS noradrenergic activity. It is speculated that the close relationship between CSF and plasma NE levels in humans is due to common control mechanisms regulating noradrenergic outflow in the CNS and periphery, functionally linking central noradrenergic systems and the peripheral SNS.

ACKNOWLEDGEMENTS

REFERENCES

1 Bennett, T., Gardiner, S.M. and Kemp, P.A., An assessment of the effectiveness of neonatal treatment with guanethidine as a means of producing sympathectomy, Br. J. Pharmacol., 76 (1982) 557–564.

2 Blythe, T.H., Hall, R.C. and Hughes, I.E., An assessment of the cardiovascular sympathectomy induced by guanethidine, J. Pharm. Pharmacol., 28 (1976) 53–57.

3 Burnstock, G., Evans, B., Gannon, B.J., Heath, J.W. and James, V., A new method of destroying adrenergic nerves in adult animals using guanethidine, Br. J. Pharmacol., 43 (1971) 295–301.

4 Chernow, B., Lake, R.C., Cruess, D., Coyle, J., Hughes, P., Balestrieri, F., Casey, L., Rainey, T.G. and Fletcher, J.R., Plasma, urine, and CSF catecholamine concentrations during and after ketamine anesthesia, Crit. Care Med., 10 (1982) 600–603.

5 Evans, M.I., Halter, J.B. and Porte, D., Jr., Comparison of double- and single-isotope enzymatic derivative methods for measuring catecholamines in human plasma, Clin. Chem., 24 (1978) 657–662.

6 Falck, B., Nielsen, K.C. and Owman, C., Adrenergic innervation of the pial circulation, Scand. J. Clin. Lab. Invest., Suppl. 102, Sect. VI (1968) B.

7 Friedman, R., Tassinari, L.M., Heine, M. and Iwai, J., Differential development of salt-induced and renal hypertension in Dahl hypertension-sensitive rats after neonatal sympathectomy, Clin. Exp. Hypertension, 1 (1979) 779–799.

8 Fuxe, K. and Ungerstedt, U., Localization of catecholamine uptake in rat brain after intraventricular injection, Life Sci., 5 (1966) 1817–1824.

9 Goldstein, D.S., Feuerstein, G., Izzo, J.L., Jr., Kopin, I.J. and Keiser, H.R., Validity and reliability of liquid chromatography with electrochemical detection for measuring plasma levels of norepinephrine and epinephrine in man, Life Sci., 28 (1981) 467–475.

10 Grzanna, R. and Coyle, J.T., Absence of a relationship between sympathetic neuronal activity and turnover of serum dopamine beta-hydroxylase, Naunyn-Schmiedeberg's Arch. Pharmacol., 304 (1978) 231–236.

11 Hartman, B.K., Zide, D. and Udenfriend, S., The use of dopamine beta-hydroxylase as a marker for the central noradrenergic nervous system in rat brain, Proc. Natl. Acad. Sci. U.S.A., 69 (1972) 2722–2726.

12 Heath, J.W., Evans, B.K., Gannon, B.J., Burnstock, G. and James, V.B., Degeneration of adrenergic neurons following guanethidine treatment: an ultrastructural study, Virchow's Arch. Cell. Pathol., 11 (1972) 182–197.

13 Holzwarth, M.A., Shinsako, J. and Dallman, M.F., Adrenal regeneration: time course, effect of hypothalamic hemi-islands and response to unilateral adrenalectomy, Neuroendocrinology, 31 (1980) 168–176.

14 Johnson, E.M. and O'Brien, F., Evaluation of the permanent sympathectomy produced by the administration of guanethidine to adult rats, J. Pharmacol. Exp. Ther., 196 (1976) 53–61.

15 Johnson, E.M., O'Brien, F. and Werbitt, R., Modification and characterization of the permanent sympathectomy produced by the administration of guanethidine to newborn rats, Eur. J. Pharmacol., 37 (1976) 45–54.

16 Kolka, M.A., Elizondo, R.S. and Weinberg, R.P., Sympathoadrenal responses to cold and ketamine anesthesia in the rhesus monkey, J. Appl. Physiol., 54 (1983) 896–900.

17 Kopin, I.J., Lake, R.C. and Ziegler, M., Plasma levels of norepinephrine, Ann. Int. Med., 88 (1978) 671–680.

18 Kvetnansky, R., Weise, V.K., Nguyen, B.T. and Kopin, J., Effects of chronic guanethidine treatment and adrenal medullectomy on plasma levels of catecholamines and corticosterone in forcibly immobilized rats, J. Pharmacol. Exp. Ther., 209 (1979) 287–291.

19 Lake, C.R., Gullner, H.G., Polinsky, R.J., Ebert, M.H., Ziegler, M.G. and Bartter, F.C., Essential hypertension: central and peripheral norepinephrine, Science, 211 (1981) 955–957.

20 Lake, C.R., Sternberg, D.E., van Kammen, D.P., Ballenger, J.C., Ziegler, M.G., Post, R.M., Kopin, I.J. and Bunney, W.E., Jr., Schizophrenia: elevated cerebrospinal fluid norepinephrine, Science, 207 (1980) 331–333.

21 Levens, N.R., Peach, M.J. and Carey, R.M., Interactions between angiotensin peptides and the sympathetic nervous system mediating intestinal sodium and water absorption in the rat, *J. Clin. Invest.*, 67 (1981) 1197–1207.

22 Madison, R., Crutcher, K.A. and Davis, J.N., Sympatho-hippocampal neurons are inside the blood–brain barrier, *Brain Research*, 213 (1981) 183–189.

23 McNicholas, L.F., Martin, W.R., Sloan, J.W. and Nozaki, M., Innervation of the spinal cord by sympathetic fibers, *Exp. Neurol.*, 69 (1980) 383–394.

24 Mefford, I.N., Ward, M.M., Miles, L., Taylor, B., Chesney, M.A., Keegan, D.L. and Barchas, J.D., Determination of plasma catecholamines and free 3,4-dihydroxyphenylacetic acid in continuously collected human plasma by high performance liquid chromatography with electrochemical detection, *Life Sci.*, 28 (1981) 477–483.

25 Moore, R.Y., Central catecholamine neuron systems: anatomy and physiology of the norepinephrine and epinephrine systems, *Ann. Rev. Neurosci.*, 2 (1979) 113–168.

26 Nielsen, K.C. and Owman, C., Adrenergic innervation of pial arteries related to the circle of Willis in the cat, *Brain Research*, 6 (1967) 773–776.

27 Oldendorf, W.H., Brain uptake of radiolabeled amino amines and hexoses after arterial injection, *Am. J. Physiol.*, 221 (1971) 1629–1639.

28 Partanen, M., Linnoila, I., Hervonen, A. and Rapoport, S.I.,The effect of aging on extra-adrenal catecholamine storing cells of the rat, *Neurobiol. Aging,* 5 (1984) 105–110.

29 Peuler, J.D. and Johnson, G.A., Simultaneous single isotope radioenzymatic assay of plasma norepinephrine, epinephrine, and dopamine, *Life Sci.,* 21 (1977) 625–636.

30 Popper, C.W., Chiueh, C.C. and Kopin, L.I., Plasma catecholamine concentrations in unanesthetized rats during sleep, wakefulness, immobilization, and after decapitation, *J. Pharmacol. Exp. Ther.*, 202 (1977) 144–148.

31 Post, R.M., Lake, C.R., Jimerson, D.C., Bunney, W.E., Jr., Wood, J.H., Ziegler, M.G. and Goodwin, F.K., Cerebrospinal fluid norepinephrine in affective illness, *Am. J. Psychiat.*, 135 (1978) 907–912.

32 Raskind, M.A., Peskind, E.R., Halter, J.B. and Jimerson, D.C., Norepinephrine and 3-methoxy-4-hydroxy phenyl glycol in cerebrospinal fluid and plasma in Alzheimer's disease, *Arch. Gen. Psychiat.*, 41 (1984) 343–346.

33 Reis, D.J. and Wurtman, R., Diurnal changes in brain noradrenalin, *Life Sci.*, 7 (1968) 91–98.

34 Skelton, F.R., Adrenal regeneration and adrenal-regeneration hypertension, *Physiol. Rev.*, 39 (1959) 162–182.

35 Smith, B.H. and Sweet, W.H., Monoaminergic regulation of central nervous system function: 1. Noradrenergic systems, *Neurosurgery*, 3 (1978) 109–119.

36 Stein, L.J., Dorsa, D.M., Baskin, D.G., Figlewicz, D.P., Ikeda, H., Frankmann, S.P., Greenwood, M.R.C., Porte, D., Jr. and Woods, S.C., Immunoreactive insulin levels are elevated in the cerebrospinal fluid of genetically obese Zucker rats, *Endocrinology*, 113 (1983) 2299–2301.

37 Weil-Malherbe, H., Axelrod, J. and Tomchick, R., Blood–brain barrier for adrenaline, *Science*, 129 (1959) 1226–1227.

38 Wilkinson, C.W., Shinsako, J. and Dallman, M.F., Return of pituitary–adrenal function after adrenal enucleation or transplantation: diurnal rhythms and responses to ether, *Endocrinology*, 109 (1981) 162–169.

39 Ziegler, M.G., Brooks, B.R., Lake, C.R., Wood, J. and Enna, S.J., Norepinephrine and gamma-aminobutyric acid in amyotrophic lateral sclerosis, *Neurology*, 30 (1980) 98–101.

40 Ziegler, M.G., Lake, C.R., Wood, J.H., Brooks, B.R. and Ebert, M.H., Relationship between norepinephrine in blood and cerebrospinal fluid in the presence of a blood–cerebrospinal fluid barrier for norepinephrine, *J. Neurochem.*, 28 (1977) 677–679.

41 Ziegler, M.G., Lake, C.R., Wood, J.H. and Ebert, M.H., Norepinephrine in cerebrospinal fluid: basic studies, effects of drugs and disease. In J.H. Wood (Ed.), *Neurobiology of Cerebrospinal Fluid*, Plenum Press, New York, 1980, pp. 141–152.