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CHOLINERGIC MECHANISMS IN THE CAT VESTIBULAR SYSTEM

I. MATSUOKA* and E. F. DOMINO

Department of Pharmacology, University of Michigan, Ann Arbor, Michigan 48104

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Summary—A neuropharmacological study was undertaken in the cat to obtain further evidence of the role of acetylcholine in vestibular function. It was shown that the vestibular nerve contains about half or less the enzymatic activity of choline acetyltransferase, acetylcholinesterase, cholinesterase and total cholinesterase than nucleus vestibularis lateralis (NVL).

Field potentials as well as discharge of single neurones of nucleus vestibularis lateralis (NVL) were studied in locally anaesthetized, decamethonium immobilized ventilated cats before, during and after vestibular nerve and reticular formation stimulation. Various cholinergic agonists and antagonists and the adrenergic agonists L-DOPA and (+)-amphetamine were then given intravenously to determine their effects. Three major evoked potentials to vestibular nerve stimulation were recorded in NVL. These potentials were labelled N_1, N_2 and N_3 on the basis of polarity and latency. Although the N_1 and N_3 waves were not much affected, the N_2 wave was dramatically enhanced by physostigmine and reduced by scopolamine.

About half of NVL neurones excited by vestibular nerve stimulation responded to muscarinic cholinergic drugs. Nucleus vestibularis lateralis responses to reticular formation (RF) stimulation were primarily nicotinic and were blocked by the nicotinic antagonist, mecamylamine, but not trimethadinium. Some neurones excited by RF stimulation were enhanced by L-DOPA or (+)-amphetamine. The data indicate that cholinergic mechanisms are strongly involved in vestibular function. Adrenergic mechanisms are also involved, but to a much lesser extent.

It is well known that anticholinergic drugs are useful antimotion remedies. Therefore, it is pertinent to determine the role of cholinergic mechanisms in vestibular function. The known histochemical localization of acetylcholinesterase (AChE) in cat nucleus vestibularis is complex. Acetylcholinesterase levels are high in the superior and lateral nuclei, but low in the medial and inferior portions (FRIEDE, 1966). In the nucleus vestibularis lateralis (NVL) the cell bodies of Deiters' giant neurones contain almost all of the AChE. SHUTE and LEWIS (1960) and ROSS (1969a, b) have also shown AChE in the vestibular ganglion of Scarpa. STEINER and WEBER (1964, 1965) and YAMAMOTO (1967) reported that NVL neurones activated by vestibular stimulation were also excited by the iontophoretic application of acetylcholine (ACh). Further neuropharmacological investigations on the cholinergic mechanisms in the vestibular system are obviously needed.

In this paper we describe our studies on the gross distribution of choline acetyltransferase (ChAc), AChE, cholinesterase (ChE) and total cholinesterase in the cat vestibular nerve and nucleus vestibularis lateralis (NVL). In addition, spontaneous unitary discharges were recorded in NVL neurones as well as field potentials to vestibular nerve stimulation before and after various cholinergic agonists and antagonists.

METHODS

Neurochemical analysis

Eight adult cats of either sex were used. After pentobarbital sodium (30 mg/kg i.v.), each animal was placed in a stereotaxic instrument. In order to locate the NVL, a small electrode was inserted into the right side of the nucleus and fixed to the cranium with dental cement. The right vestibular nerve and brainstem were exposed under microscopic surgery using a Zeiss microscope (X6-X40). The vestibular nerve including Scarpa's ganglion and NVL was dissected out for chemical assay. Choline acetyltransferase was assayed radiochemically using a modification of the method of SCHRIER and SCHUSTER (1967) and

* Present address: Department of Otolaryngology, Faculty of Medicine, University of Kyoto, Kyoto, Japan.

McCAMAN and HUNT (1965). Total ChE, AChE and ChE were assayed radiochemically using the method of SIAKATOS, FILBERT and HESTER (1969). Total protein was assayed by the method of LOWRY, ROSEBROUGH, FARR and RANDALL (1951).

Neuropharmacological studies

Thirty-nine normal adult cats of either sex, weighing 2·5–4·0 kg, were used. All surgery was performed under diethyl ether–oxygen anaesthesia. The trachea, femoral artery and vein were cannulated. A section, about 8 mm diameter, of the mucous membrane of the middle ear cavity was removed through an approach via the right tympanic bulla. A small concentric bipolar steel electrode was inserted through the round window according to the method of FREDRICKSON, SCHWARTZ and KORNHUBER (1966). Another electrode was stereotaxically inserted through the cerebellum into the reticular formation (RF). The recording microelectrodes were gold tipped stainless steel and tungsten wires (BOUDREAU, BIERER and KAUFMAN, 1968; HUBEL, 1957; NAITO, Personal communication). Their electrical resistance was between 0·75 and 10·0 MΩ. With reference to a stereotaxic brain atlas (SNIDER and NIEMER, 1961), the recording electrode was inserted into the right vestibular nucleus (MATSUOKA, 1967, 1969), primarily NVL. The microelectrode was connected through a cathode follower to a Grass P-5 amplifier and the potentials displayed on a Tektronix dual beam oscilloscope. An amplitude discriminator was used to convert the action potentials into a pulse of constant amplitude and duration (TMC, Model 605, 606). This was fed into a CAT 400B computer and data printer (Model 500A) which yielded on-line poststimulus time histograms of single unit activity.

In each recording, 30 sweeps following stimulation or spontaneous unit activity were summated on the computer. All data displayed on the oscilloscope were photographed on Kodak plus X film using a Grass recording camera. In some cases, the data from the computer were also displayed on a dual beam oscilloscope and recorded on Polaroid film.

After all operative procedures were completed, the animal was locally anaesthetized at all wound edges with 0·5% lidocaine and then immobilized with decamethonium (1·0 mg/kg per hr i.v.). Artificial respiration was maintained immediately after injection of decamethonium. Body temperature was kept at 36–38°C by means of an automatic heating pad (German Rupp Industry Inc., Model K-1-3). Under these conditions, the mean ± S.E. values of pH, PO_2 and PCO_2 of arterial blood of 5 animals were as follows: pH 7·37 ± 0·005, PO_2 = 105·0 ± 5·7 mm Hg, and PCO_2 = 39·0 ± 6·0 mm Hg. These values were almost the same as normal control animals. The arterial blood pressure was recorded by means of a strain gauge from the femoral artery.

The right vestibular nerve and the RF were stimulated with a square wave pulse, 0·05 msec and 0·1 msec in width, delivered from a Grass S-8 stimulator. The intensity of stimuli ranged from 1·0 to 5·0 V. Single shock stimuli were usually used, but in some cases pulse trains (50, 150 and 250 Hz, 40 msec train duration and 0·1 msec pulse width) were applied. At the end of each experiment, a 22·5 V d.c. current was applied to the microelectrode so that the site of recording could be confirmed histologically (see Fig. 1).

RESULTS

Cholinergic neurochemistry of the vestibular system

As shown in Table 1, the content of ChAc, AChE, ChE and total ChE was higher in NVL than in the vestibular nerve including Scarpa's ganglion.

Field potentials in NVL to electrical stimulation of the ipsilateral vestibular nerve

Field potentials evoked to vestibular nerve stimulation were classified as N_1, N_2 and N_3 waves. These potentials were almost the same as the P, N_1 and N_2 waves of SHIMAZU and PRECHT (1965). The N_1 wave was a small negative and sometimes positive potential. The N_2 wave was a large sharp negative potential and the N_3 wave was a wide negative potential. The means ± S.E. of the latency on these three waves for seven animals were N_1 = 0·50 ± 0·03, N_2 = 1·09 ± 0·03, and N_3 = 2·43 ± 0·21 msec. These values were almost the same as those obtained by SHIMAZU and PRECHT (1965). When the microelec-

Table 1. Cholinergic neurochemistry of the cat vestibular axon and nucleus vestibularis lateralis

Assay	n	Axon	n	Nucleus
ChAc	6	2·78 ± 0·77	6	4·30 ± 0·65
AChE	2	78·50 ± 43·51	7	171·94 ± 27·13
ChE	3	75·67 ± 46·76	7	280·20 ± 37·04
Total ChE	4	262·90 ± 149·10	7	939·40 ± 142·20
Protein	4	0·75 ± 0·15	7	0·85 ± 0·09

ChAc is expressed as mean μmol ACh/g per hr ± S.E.
AChE was measured using ^{14}C-labelled methacholine, ChE using [^{14}C]-butyrylcholine and total ChE using [^{14}C]-ACh as substrates and expressed as mean μmol/g per hr. Protein is expressed as mg/ml of 1% homogenate.

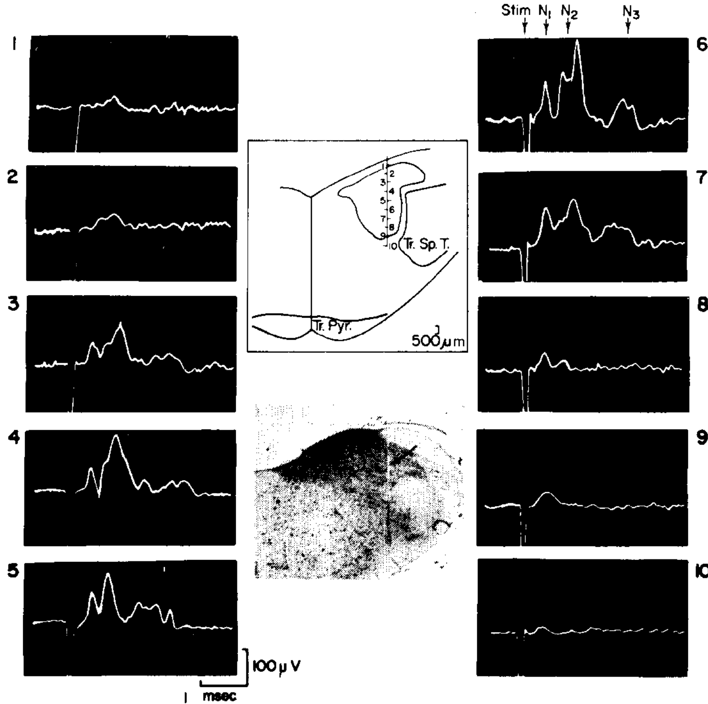

Fig. 1. Evoked potential responses of nucleus vestibularis lateralis to ipsilateral vestibular nerve stimulation. Tr. Pyr.: tractus pyramidalis: Tr. sp. T.: tractus spinalis n. trigemini. Evoked potentials at varying depths in NVL are illustrated. Note that following the stimulus artifact, prominent N_1, N_2 and N_3 waves are recorded at positions 3–7 in this nucleus. Note that the evoked potential of N_2 wave has two peaks at position 6.

trode was inserted into NVL, a characteristic field potential was evoked by single shocks to the vestibular nerve. The typical response was always elicited in the middle portion of NVL. The N_2 wave sometimes had two or three peaks. This indicates that there are different conducting axons in the peripheral vestibular nerve. The alterations in the N_1 and N_2 waves during different placements of the microelectrode from a dorsal to a ventral trajectory are shown in Figure 1. The potentials are helpful for locating single neuronal responses to specific portions of NVL. The mean amplitudes ± S.E. of these waves in seven animals are as follows: N_1 = 96·7 ± 10·7, N_2 = 176·3 ± 14·5 and N_3 = 123·4 ± 17·5 μV, as illustrated in the bar graph in Figure 2.

Effects of cholinergic agonists

Two minutes after administration of nicotine (25 μg/kg i.v.), the mean amplitude of the N_1 wave was not significantly reduced ($P > 0·05$) to 73·5 ± 10·5 μV. The mean amplitude of the N_2 wave was significantly reduced to 101·2 ± 36·1 μV ($P < 0·01$) while the amplitude of the N_3 wave did not change ($P > 0·05$). Five minutes after administration of physostigmine (25 μg/kg i.v.), the mean amplitude of the N_2 wave increased to 315·0 ± 29·8 μV ($P < 0·001$). However, the N_1 and N_3 waves did not change (Fig. 2). The accumulative

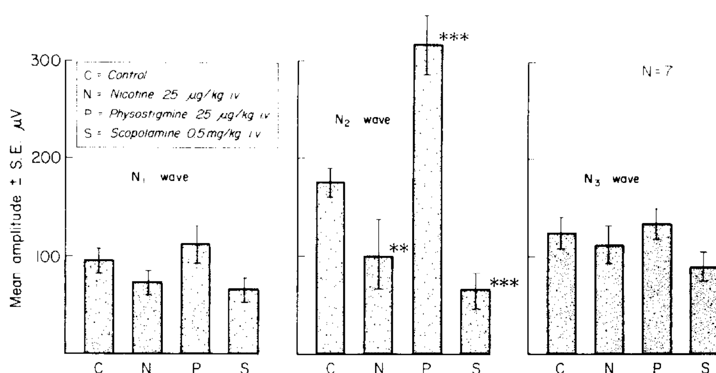

Fig. 2. Effects of various cholinergic drugs on the amplitude of evoked nucleus vestibularis lateralis potentials elicited with single shocks to the ipsilateral vestibular nerve. Each bar height represents the mean amplitude of a specific wave as designated. Note the dramatic effects of physostigmine in facilitating the N_2 wave as well as scopolamine in reducing it. This wave was reduced following nicotine. All responses were measured 5 min after drug administration except after nicotine, which was measured 2 min after injection. * = $P < 0{\cdot}05$; ** = $P < 0{\cdot}01$; *** = $P < 0{\cdot}001$.

dose response curve of physostigmine is shown in Figure 3. The N_1 wave was not significantly increased with intravenous doses of 25 and 50 μg/kg. The N_2 wave was remarkably enhanced even in small doses (5 μg/kg i.v.). The N_3 wave did not change at all. The optimal intravenous dose of physostigmine which facilitated the N_2 wave was 25 μg/kg. Larger doses caused a slight reduction.

Effects of cholinergic antagonists

As described above, the amplitude of the N_2 wave was enhanced by physostigmine in intravenous doses of 25 μg/kg. This enhancement was reduced by scopolamine (0·5 mg/kg i.v.). In this dose, scopolamine did not reduce the N_1 and N_3 waves. The effects of cumulative doses of scopolamine are shown in Figure 3. The N_1 wave was also reduced by large doses of scopolamine. In contrast, the N_2 wave was markedly reduced, even with small doses of scopolamine (0·25 mg/kg i.v.).

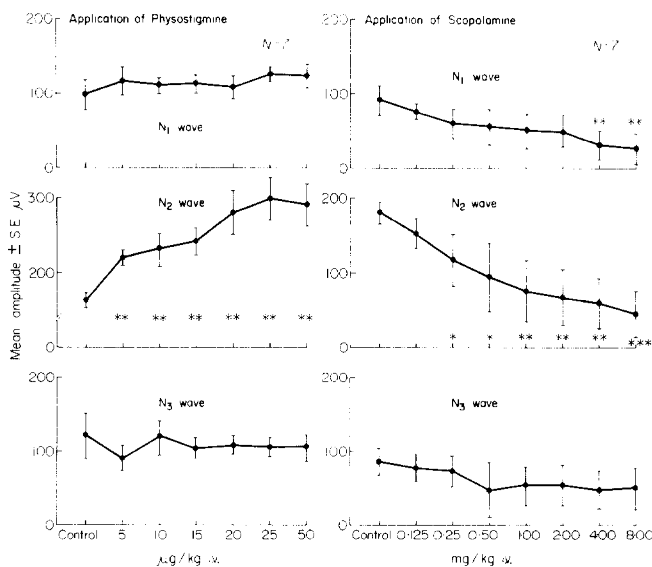

Fig. 3. Accumulative dose effect curves of the action of physostigmine and scopolamine on nucleus vestibularis lateralis field potentials. Drugs were administered intravenously in an accumulative dose fashion every 5 min. Note the dramatic effects of physostigmine and scopolamine on the N_2 wave in particular. Very large doses of scopolamine depress the N_1 potential, suggesting a possible presynaptic influence. Probability as in Figure 2.

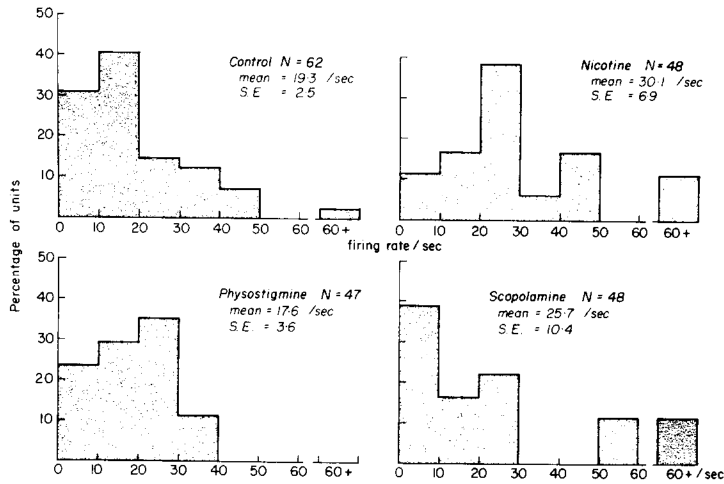

Fig. 4. Modification of the mean firing rate of nucleus vestibularis lateralis neurones following various cholinergic drugs. Note that scopolamine and nicotine tended to produce a bimodal distribution of neuronal firing.

Spontaneous unitary responses of NVL

The spontaneous unitary discharges from NVL were recorded mainly as biphasic but sometimes as monophasic spikes. The majority of units in NVL were 0·5–1·5 mV in amplitude which discharged at a rate of 0·1–80 Hz. The mean ± S.E. firing rate of 62 units was 19·3 ± 2·5/sec, as illustrated in the bar graph in Figure 4. This value is slightly lower than in other studies (MATSUOKA, 1967, 1969). Nicotine (25 μg/kg i.v.) and scopolamine (0·5 mg/kg i.v.) caused slightly greater spontaneous discharge rates. Physostigmine (25 μg/kg i.v.) did not change the distribution of spontaneous discharge.

Response of NVL neurones to vestibular nerve stimulation

Trains of stimuli of 40 msec duration, 0·05–0·1 msec pulses at 50, 150 and 250 Hz were applied to the ipsilateral vestibular nerve. Optimal responses in NVL units were obtained at 250 Hz. This frequency also produced excellent unit responses to RF stimulation. Thirty-eight neurones were studied which responded to ipsilateral vestibular nerve stimulation. Twenty-two NVL neurones excited by vestibular nerve stimulation were further stimulated by physostigmine (25 μg/kg i.v.). The excitant effects of physostigmine were antagonized by scopolamine (0·5 mg/kg i.v.), as shown in Figure 5. The discharge rate of

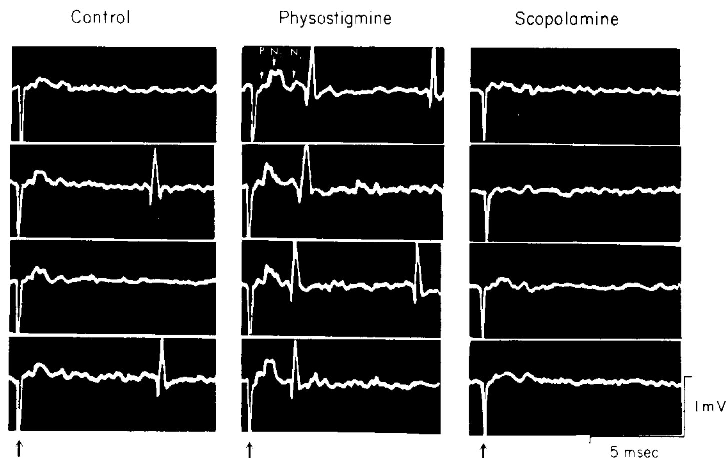

Fig. 5. Effects of physostigmine and scopolamine on unit discharge of nucleus vestibularis lateralis neurones. The ipsilateral vestibular nerve was stimulated with single shocks of 0·05 msec duration and 2·5 V. Negativity is upward. Time base and voltage calibration are as illustrated. Note that physostigmine tends to facilitate single unit discharge while scopolamine depressed it.

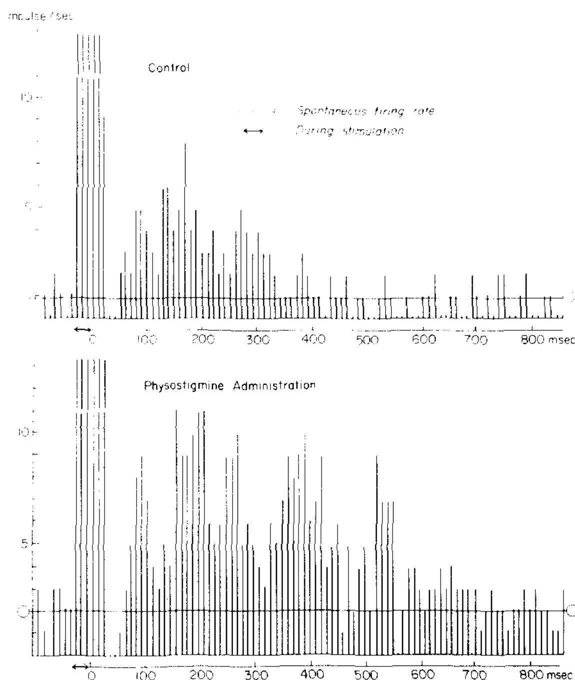

Fig. 6. Post-stimulus time histogram of responses of single nucleus vestibularis lateralis neurones to ipsilateral vestibular nerve stimulation before and after physostigmine. Note the enhanced effects following physostigmine in an intravenous dose of 25 μg/kg. The vestibular nerve was stimulated with a 40 msec train of stimuli 0·1 msec pulses, 3·0 V, 250 Hz. Each ordinate represents 10 msec and is the mean of 30 stimuli.

these neurones was not changed by nicotine (25 μg/kg i.v.). The discharge rate of five NVL neurones excited by vestibular nerve stimulation was depressed by physostigmine and enhanced by scopolamine. Four of these units were not affected by nicotine. Twenty-seven neurones excited by vestibular nerve stimulation did not change their rate of discharge following nicotine. Three neurones excited by vestibular nerve stimulation but not enhanced by physostigmine showed enhanced discharges following (+)-amphetamine in intravenous doses of 0·5 mg/kg. Two NVL units excited by physostigmine were unaffected by L-DOPA (10 mg/kg i.v.). Only three neurones inhibited by vestibular nerve stimulation were stimulated by nicotine (25 μg/kg i.v.). The effects of nicotine were completely antagonized by 2·0 mg/kg mecamylamine given intravenously (see Table 2). In view of the fact that nicotine caused a reduction of the N_2 potential, these three NVL neurones might be inhibitory. Trains of stimuli to the vestibular nerve were always more effective than single shocks. Normally short trains of stimuli produced effects lasting about 200 msec. After physostigmine, these effects were markedly prolonged (about 500–600 msec), as illustrated in Figure 6.

Table 2. Nucleus vestibularis lateralis unit responses to vestibular nerve stimulation

Control response to vestibular stimulation	Physostigmine 25 μg/kg	Scopolamine 0·5 mg/kg	Nicotine 25 μg kg	Mecamylamine 2·0 mg/kg	Trimethidinium 1·0 mg/kg	L-DOPA 10·0 mg/kg	(+)-Amphetamine 0·5 mg kg	Number of units
	Enhanced	Depressed	No change					20
	Depressed	Enhanced						1
	Depressed	Enhanced	No change					4
Excited	No change	No change					Enhanced	3
			No change				Enhanced	1
			No change		No change			2
	Enhanced					No change		2
	No change					Enhanced		2
Inhibited			Enhanced	Depressed				3

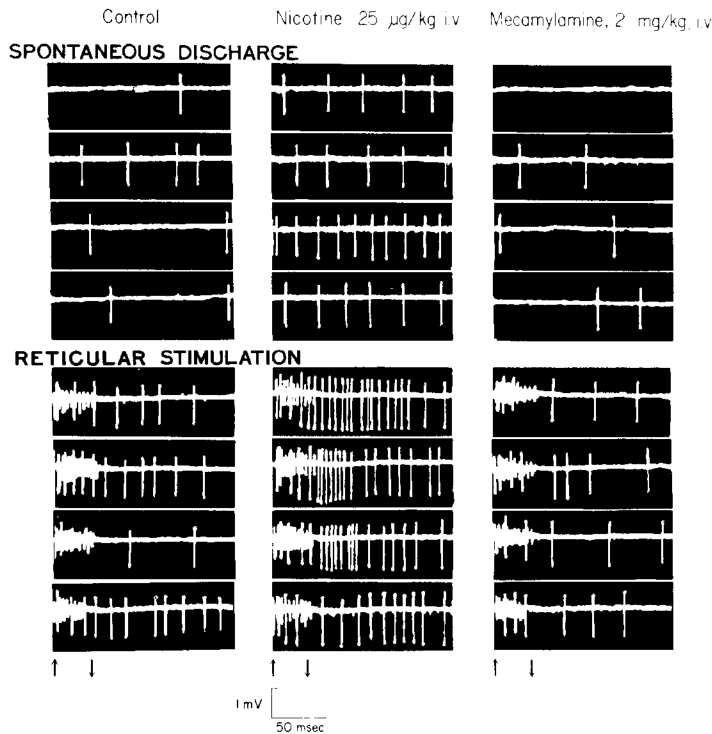

Fig. 7. Modification of nucleus vestibularis lateralis neurone discharge to reticular stimulation by nicotine and mecamylamine. Note that nicotine caused a marked facilitating effect in both the spontaneous and evoked rates of unit discharge. These effects were diminished by mecamylamine. Neuronal firing to trains of stimuli to the reticular formation, however, were not greatly affected. Stimulus parameters as in Figure 6.

Responses of NVL neurones to RF stimulation

Thirty-seven NVL neurones were studied before and after RF stimulation. The responses to single RF shocks usually lasted about 10–50 msec. Nucleus vestibularis lateralis unit responses to a train of stimuli were always greater and more prolonged than with single stimuli. Sixteen neurones excited by RF stimulation were even further stimulated following physostigmine (25 µg/kg i.v.). Scopolamine (0·5 mg/kg i.v.) did not depress the effects of reticular stimulation. Thirteen units excited by reticular stimulation were further stimulated by 25 µg/kg nicotine. Of these, eight were depressed by physostigmine and five were stimulated by scopolamine. Three were not affected by scopolamine but were enhanced by L-DOPA and/or (+)-amphetamine (see Figure 7 and Table 3). Twenty-five neurones excited by RF stimulation were further stimulated by nicotine. Six of these were depressed by mecamylamine (2·0 mg/kg i.v.) (see Fig. 8). However, in another six units the effects of nicotine were not altered by pretreatment with trimethidinium (1·0 mg/kg i.v.). Only one NVL neurone did not respond to either RF stimulation or the intravenous administration of cholinergic agonists.

Table 3. Nucleus vestibularis lateralis unit responses to RF stimulation

Control response to RF stimulation	Physostigmine 25 µg/kg	Scopolamine 0·5 mg/kg	Nicotine 25 µg/kg	Mecamylamine 2·0 mg/kg	Trimethidinium 1·0 mg/kg	L-DOPA 10·0 mg/kg	(+)-Amphetamine 0·5 mg/kg	Number of units
	Enhanced	No change	Enhanced					13
	Enhanced	No change				No change		3
	Depressed	Enhanced				Enhanced		5
Excited	Depressed	No change				Enhanced	Enhanced	3
			Enhanced	Enhanced				6
			Enhanced		No change			6
No change	No change		No change					1

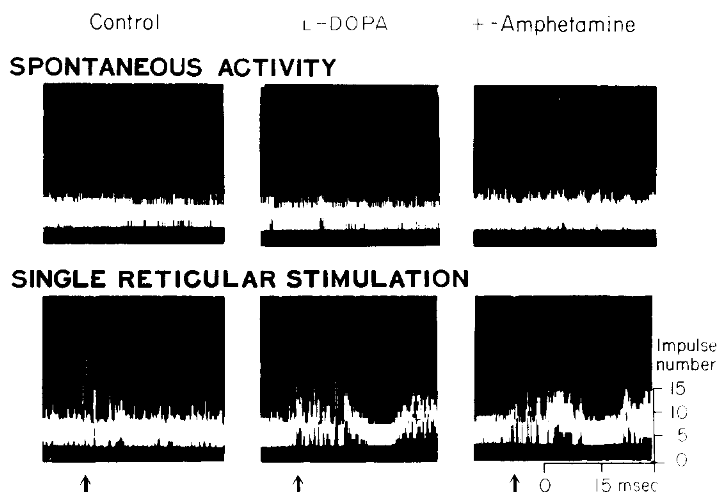

Fig. 8. Post-stimulus histogram of responses of single nucleus vestibularis lateralis neurones to the administration of L-DOPA and (+)-amphetamine. The effects were recorded approximately 5 min after administration of 10 mg/kg L-DOPA and 1 mg/kg (+)-amphetamine, intravenously. Note that L-DOPA and (+)-amphetamine markedly enhanced unit discharge following single reticular stimuli given at the arrows.

DISCUSSION

MICKLE and ADES (1954) recorded evoked responses in NVL to ipsilateral vestibular nerve stimulation. These potentials consisted of irregular waves of 1·0–1·25 msec latency. Subsequently, GERNANDT, IRANYI and LIVINGSTON (1959) reported similar evoked responses with a latency of less than 1·0 msec. More recently, PRECHT and SHIMAZU (1965); SHIMAZU and PRECHT (1965, 1966) have used microelectrode techniques for recording field potentials elicited by ipsilateral vestibular nerve stimulation. They described an initial positive to negative P wave, a large sharp negative N_1 wave and a delayed negative N_2 wave. The P wave had a latency of 0·66 msec and was interpreted as indicating the arrival of the afferent presynaptic impulse. The N_1 wave with a latency of 1·06 msec and a duration of 1·0 msec was attributed to monosynaptically evoked spikes of vestibular neurones. The N_2 wave which had a latency of 2·4 msec was attributed to polysynaptically evoked spikes. The P, N_1, and N_2 waves of SHIMAZU and PRECHT correspond to our N_1, N_2, and N_3 waves. In our data the N_1 wave was most often a small negative potential (hence N) with a mean latency ± S.E. of 0·50 ± 0·03 msec. The N_2 wave was a sharp, large negative potential with a mean latency ± S.E. of 1·09 ± 0·03 msec. The N_3 wave was a broad negative wave with a mean latency ± S.E. of 2·41 ± 0·21 msec. These values are almost the same as those of SHIMAZU and PRECHT. The N_1 and N_3 waves were not altered by either cholinergic agonists or antagonists except that a large dose of 4·0 mg/kg of scopolamine reduced the N_1 wave. The N_2 wave attributed to monosynaptically evoked units was dramatically enhanced by physostigmine and antagonized by scopolamine.

STEINER and WEBER (1964, 1965) have reported that two-thirds of Deiters' neurones are facilitated by the iontophoretic application of ACh. SALMOIRAGHI and STEFANIS (1965) have also reported that Deiters' neurones are consistently facilitated by both ACh and norepinephrine (NE). YAMAMOTO (1967) has also reported that NVL neurones are excited by iontophoretically applied ACh and NE and depressed by atropine. His data on ACh and atropine agree with our data, i.e. NVL neurones excited by vestibular nerve stimulation were further facilitated by physostigmine and depressed by scopolamine, but were not stimulated by nicotine. Nucleus vestibularis lateralis neurones excited by RF stimulation were enhanced in their response by nicotine and blocked by the central and peripherally acting nicotinic cholinergic antagonist, mecamylamine. Pretreatment with the peripherally acting nicotinic cholinergic antagonist, trimethidinium, had no effect on the nicotine response, suggesting a central action of nicotine.

The latency of onset with iontophoretic application of NE to NVL cells takes as long as 60 sec or more (YAMAMOTO, 1967). One would expect a much shorter latency for a chemical transmitter. These findings suggest that NVL neuronal responses to direct application of NE involves slower, perhaps indirect, mechanisms. It should be noted that none of the vestibular nuclei contain many catecholamines (DAHLSTRÖM and FUXE, 1964a, b, 1965; FEX, FUXE and LENNERSTRAND, 1965; FUXE, 1965).

FELDBERG and VOGT (1948) measured the rate of ACh synthesis in the isolated vestibular nerve and nucleus. The rate of ACh synthesis in the vestibular nucleus was higher than in its peripheral nerve (70 μg/g versus 49 μg/g). Our own values of 4·30 versus 2·78 μmol/g per hr for ChAc activity are thus qualitatively similar. The distribution of AChE using the histochemical technique of KOELLE (1951) has been described in the vestibular nerve, ganglion of Scarpa, and cells of the vestibular nuclei (LEWIS and SHUTE, 1967; OSEN and ROTH, 1969; SHUTE and LEWIS, 1960). Again our data (Table 1) is in basic agreement. In general, NVL contains more of these enzymes than the vestibular nerve, whether expressed per gram of wet tissue or per gram of protein per millilitre of homogenate.

The amplitude of the NVL presynaptic potential due to vestibular stimulation did not change following physostigmine and scopolamine. It appears that the vestibular nerve is not cholinergic, although it does contain some cholinergic enzymes. According to our pharmacological data, the primary vestibular afferent cannot be cholinergic. The relatively low content of AChE in the vestibular nerve might be explained by the data of ROSS (1969a, b) who observed that the AChE of the vestibular nerve is not in the vestibular primary afferent, but in an autonomic nervous system neurone in Scarpa's ganglion. The major criticism of the present research is that the changes induced by the drugs studied may be reflex in origin and not directly on the vestibular nucleus. Further studies involving the direct application of these agents to NVL neurones is clearly indicated.

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