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EFFECTS OF NICOTINE AND SOME NICOTINE-LIKE COMPOUNDS INJECTED INTO THE CEREBRAL VENTRICLES OF THE CAT

BY

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The pharmacological actions of nicotine on the central nervous system have been studied for many years (Silvette, Hoff, Larson & Haag, 1962 ; Larson & Silvette, 1965). Little attention, however, has been paid to the minimal dose of nicotine required to produce the particular effect under investigation and it has not always been established that the nicotine is in fact acting directly on the central nervous system. The present paper describes some of the effects of nicotine injected directly into the cerebral ventricles of conscious and anaesthetized cats. One of the regular effects of nicotine injected by this route in amounts ranging from 1–100 μg (base) was vigorous twitching of the ears. This was first reported by Armitage, Milton & Morrison (1965) and Hall & Reit (1965). The effects of ganglion-blocking drugs and cholinesterase inhibitors on the ear twitching induced by nicotine have been studied and the important observation has been made that cholinesterase inhibitors can potentiate this action of nicotine.

METHODS

Cats of either sex weighing between 2.5 and 3.5 kg were used for the experiments in which injections were made into one of the lateral ventricles. A Collison cannula, first described by Feldberg & Sherwood (1953), was screwed into the skull through a hole which was drilled and tapped 5–6 mm from the coronal suture and 3–4 mm from the midline. The lower end of the cannula shaft was made of polythene tubing. A hole was made in this tubing with a cautery near the end and the tip was sealed off with a drop of araldite. Drugs were injected into the ventricular cannula in a volume of 0.2 ml. through one arm of a " Y " shaped piece of polythene tubing. This was attached to a No. 18 hypodermic needle which was pushed through the rubber diaphragm. 0.2 ml. artificial c.s.f. (Carmichael, Feldberg & Fleischhauer, 1964) was then injected from the second arm, so that no drug was left in the cannula. The whole injection procedure took 15 sec and was used for experiments on conscious cats and on cats anaesthetized with chloralose (70–80 mg/kg i.v.) or pentobarbitone sodium (25 mg/kg i.v.). Observations were made on 12 conscious cats, 42 anaesthetized cats, two spinal cats and two decerebrate cats. Cats were made spinal under ether anaesthesia by cutting the cord at the level of the second cervical vertebra. The brain was then destroyed by pushing a small rod through the foramen magnum. Decerebration was performed under ether anaesthesia as described by Burn (1952).

Ear twitches were recorded in anaesthetized cats either by means of a lightly weighted frontal writing lever or a spring-loaded heart lever writing on a kymograph.

Drugs were administered either intraventricularly as described above, intravenously into a femoral vein (anaesthetized cats) or into the cephalic vein (conscious cats), or by topical application to the dorsal surface of the cervical cord, exposed by removing the occipital bone and atlas. Drugs were applied to the cord at the level of the atlanto-occipital membrane either by means of a slow continuous drip at the rate of 0.2 ml./min using a Braun perfusion pump or by application of a cotton wool swab saturated with the appropriate drug solution. In a few experiments, single injections were made through a small hole in the membrane without exposing the cord. Blood pressure was recorded from a femoral artery using a mercury manometer.

Nicotine solutions for intraventricular injection or for direct application to the spinal cord were made up immediately before use by diluting a concentrated solution of nicotine acid tartrate with artificial c.s.f. The *p*H of artificial c.s.f. at 20° was 8.2; the *p*H of a 100 μg/ml. solution of nicotine acid tartrate in artificial c.s.f. was 7.3.

The following drugs were used: nicotine hydrogen tartrate, mecamylamine hydrochloride, hexamethonium bromide, neostigmine bromide, physostigmine sulphate, nicotine monomethiodide, dimethylphenylpiperazinium iodide (DMPP), nornicotine, anabasine and metanicotine, the last three drugs all being made up as tartrate. All doses are expressed in terms of base.

RESULTS

Conscious Cats

Nicotine injected into either the left or right lateral ventricle in doses ranging from 1–100 μg, regularly produced twitching of the ears, which usually started within 10–20 sec of the injection, salivation, licking, retching and vomiting. Twitching was sometimes so rapid that the ears were folded back in the state of retraction and merely fibrillated. Most cats retired to a corner of the cage and sat there in a characteristic crouching posture. Violent head shaking was sometimes observed. With the higher doses (20–100 μg) there was panting, inability to stand and unresponsiveness to noise and touch. Some cats urinated and defaecated. The animals had usually recovered fully within 30 min.

Feldberg & Fleischhauer (1965) have listed the numerous effects caused by the injection of different substances into the cerebral ventricles of unanaesthetized cats. The most striking effect of nicotine, and the only one which was not described by Feldberg & Fleischhauer (1965) for any other substance, was the continuous twitching of the ears. This closely resembled repeated activation of the pinna reflex. Other nicotine-like compounds were tested for their ability to cause ear twitching and the results of these experiments on two cats are summarized in Table 1.

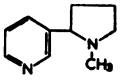
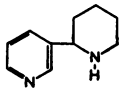
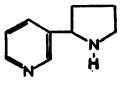
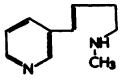
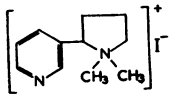
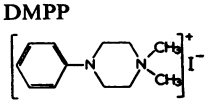
The rapid twitching of the ears produced by nicotine started 10 to 20 sec after the injection and reached a maximum speed within the next few sec. With the other compounds the onset was usually delayed and the maximum speed of twitching not reached for several minutes. The ear twitching caused by anabasine and metanicotine was never as fast as that caused by nicotine. Metanicotine, nicotine monomethiodide and DMPP induced a sporadic ear twitching which never reached a steady rate. Some of the other symptoms which are typical of nicotine and related compounds are included in Table 1. Salivation, licking, vomiting and retching were more pronounced after nicotine and anabasine than after the other compounds.

The threshold dose of nicotine required to induce ear twitching varied considerably in different cats. In cats 1 and 2 of Table 1, in which the threshold was particularly

TABLE 1

EFFECTS OF INTRAVENTRICULAR INJECTIONS OF NICOTINE AND SOME NICOTINE-LIKE COMPOUNDS ON TWO CONSCIOUS CATS

Ear twitching, vomiting, and salivation: intensity shown as + to +++, 0 = no effect. Panting, rapid breathing and eye closure: * symptom present; † symptom absent

		Ear twitching								
Drug	Dose (μg/cat)	Cat	Intensity	Onset (sec after injection)	Duration (min)	Vomiting	Salivation and licking	Panting	Rapid breathing	Eyes closed
Nicotine 	2·5	1	+++	40	12	+	+	†	†	†
		2	+++	20	13	++	+	†	*	†
	5·0	1	+++	5	15	++	+	†	†	†
		2	+++	25	10	++	+	†	*	†
	20·0	1	+++	5	20	++	++	*	*	†
Anabasine 	10·0	1	+	15	5½	+	+	†	†	†
	20·0	1	++	70	20	++	+	†	†	†
		2	+	120	8½	++	++	*	*	†
Nornicotine 	20·0	1	++	105	29	+	+	†	*	†
		2	++	105	15	+	+	†	†	†
Metanicotine 	10·0	1	0	0	0	0	0	†	†	†
	20·0	1	+	110	15	0	0	†	†	*
		2	+	130	15	0	0	†	*	†
Nicotine monomethiodide 	20·0	1	+	60	24½	0	0	†	†	†
		2	+	280	8	0	0	†	*	†
DMPP 	50·0	1	+	120	7½	0	0	†	†	*
		2	0	0	0	0	0	†	†	*

low, 50 μg DMPP had little effect; the cats lay down quietly with their eyes closed. Sporadic ear twitching occurred in only one of them. Immediately following the intraventricular injection of larger doses of DMPP (100 μg or 150 μg), there was loud calling, sometimes followed by head shaking, tail lashing, spitting and collapse. When these symptoms subsided, the cats sat down quietly with their eyes closed.

On intravenous injection of nicotine, 20 μg/kg was either ineffective or caused slight ataxia and twitching of the ears for a few seconds; recovery was complete in about 2 min. Three cats which were given 40 μg nicotine/kg all became ataxic and there was vigorous ear twitching for a short period. In two cats there was collapse and rapid breathing and in one slight salivation. Recovery occurred in 5–7 min. A dose of 60 μg nicotine/kg was given to one cat. It caused a mild convulsion within a few sec of the injection. The cat then became very ataxic, it panted, salivated profusely and the ears twitched rapidly for about a minute. Afterwards the cat was subdued and inactive but it recovered fully within 15 min. Vomiting and retching, which always occurred after an intraventricular injection of a small amount of nicotine, was not observed in any of the cats.

In one cat (Cat 1 of Table 1) effects of the ganglion-blocking drugs mecamylamine and hexamethonium were investigated on the ear twitching induced by an intraventricular injection of 5 μg nicotine. In 5 control experiments the ear twitching lasted from 11½ to 17½ min (mean 14½ min). Thirty min after an intravenous injection of 0.15 mg/kg of mecamylamine the nicotine-induced twitching lasted 8½ min. Two minutes after 0.2 and 0.5 mg/kg of mecamylamine it lasted 5½ and 6½ min respectively and the rate of twitching was greatly reduced. There was also less vomiting and retching. Each of these ganglion blocking drugs, in a dose of 0.4 mg/kg, was injected intravenously 2½ min after intraventricular nicotine. After mecamylamine, ear twitching persisted for only 5 min whereas after hexamethonium it continued for 14½ min. When hexamethonium (200 μg) was injected intraventricularly 10 min before the nicotine, the ear twitching was abolished.

Anaesthetized cats

In cats anaesthetized with chloralose or pentobarbitone sodium, an intraventricular injection of 20 μg nicotine generally caused salivation, laboured respiration and a burst of ear twitching, comparable to that caused by 2.5–5 μg in a conscious animal. Subthreshold doses of nicotine sometimes facilitated the pinna reflex. There was usually a fall in arterial blood pressure within 30 sec of the injection, but in some experiments there was a rise and in some a biphasic response. When the intraventricular injections of nicotine were repeated more than once every 30–60 min, there was total or partial block of the ear response.

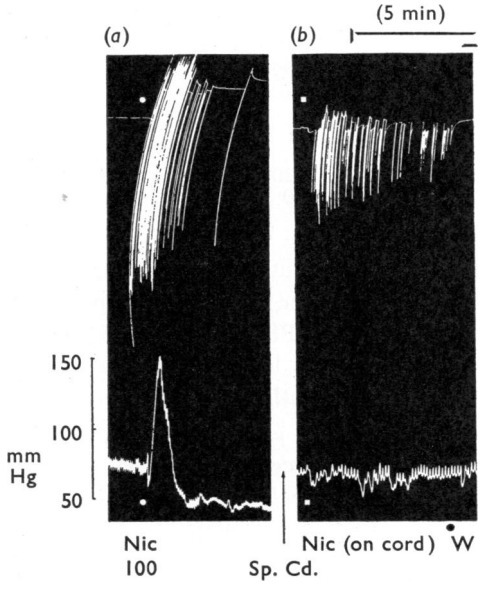


Fig. 1. Cat, 2.6 kg, decerebrate. Upper trace: record of left ear twitches. Lower trace: record of femoral blood pressure. Nic=nicotine, with the dose as μg/kg injected intravenously at the white dot. Between (*a*) and (*b*) the upper cervical cord was exposed (Sp.Cd.). At the white square, a cotton-wool swab soaked in a solution containing 2×10^{-5} g/ml. of nicotine was applied to the cord for 6 min. Time interval between (*a*) and (*b*), 45 min.

Twitching of the ears was also induced by larger amounts of nicotine injected intravenously. In some cats 40 μg/kg was sufficient to cause ear twitching; in others this amount of nicotine was ineffective and ear twitching did not occur until the dose was raised to 150 μg/kg. Nicotine also produced ear twitching when applied topically to the upper cervical cord. The lowest concentration of nicotine necessary to induce ear twitching by topical application varied from 10^{-5} to 10^{-4} g/ml.

Transection of the cervical cord. Intravenous injections of nicotine did not cause ear twitching in the spinal cat even when the dose was increased until the pressor response was abolished. Transection of the cord at the level of the second cervical vertebra in the chloralose anaesthetized animal, however, did not prevent the ear twitching produced by nicotine injected intravenously or intraventricularly, or applied to the cord. The ear response no longer occurred when the transection was at the level of C1 or above.

Decerebration. Decerebration did not affect the ear twitching induced by nicotine injected intravenously or applied to the cord, as shown in Fig. 1.

Ganglion-blocking drugs. Intravenous injections of hexamethonium (0.4 mg/kg or more) usually did not affect the ear twitching produced by intravenous or intraventricular nicotine. This is illustrated in Fig. 2. In this experiment 40 μg/kg of nicotine given intravenously had not produced ear twitching. In other experiments the ear response was sometimes reduced or abolished, but only when the nicotine was administered at least 60 min after the hexamethonium. The rise in blood pressure caused by an intravenous injection of nicotine and the fall caused by an intraventricular injection were always greatly reduced or abolished immediately following the injection of hexamethonium. Intravenous mecamylamine or pempidine, on the other hand, immediately prevented or abolished the ear twitching induced by nicotine. This antagonism between nicotine and mecamylamine is illustrated in Figs. 2 (d) and 3. On intraventricular injection of 20–50 μg all three ganglion-blocking substances abolished the nicotine-induced ear twitching within a few minutes of their application.

Atropine. An intravenous injection of 1 mg/kg, which blocked the vasodilator action of acetylcholine, had no effect on nicotine-induced ear twitching. Atropine was also without effect when injected intraventricularly in amounts up to 250 μg.

Cholinesterase inhibitors. An intravenous injection of physostigmine potentiated the ear response both in intensity and duration. A typical experiment is illustrated in Fig. 4. In contrast, intravenous neostigmine did not potentiate the ear response. When injected intraventricularly in amounts of 10 or 20 μg, however, it potentiated the ear twitching produced by a subsequent intraventricular injection of nicotine in three out of six experiments. One of these is illustrated in Fig. 5. Another is summarized in Table 2 in order to show the duration of the ear twitching before, 20 min and approximately 60 and 120 min after the neostigmine injection. After 60 min, the nicotine-induced ear twitching was still prolonged but after 120 min it had returned to the control figure.

Five experiments were performed to see whether physostigmine in a concentration of 10^{-5} or 2×10^{-5} g/ml., when perfused over the dorsal surface of the exposed cervical cord, potentiated the ear twitching induced by nicotine. Nicotine in a concentration of 10^{-5}–10^{-4} g/ml. was applied to the cord, either for 2 min with a saturated cotton-wool swab or for 30 sec by a slow continuous drip. Thirty or 60 min after the physostigmine application the ear twitching in response to nicotine was more rapid during the

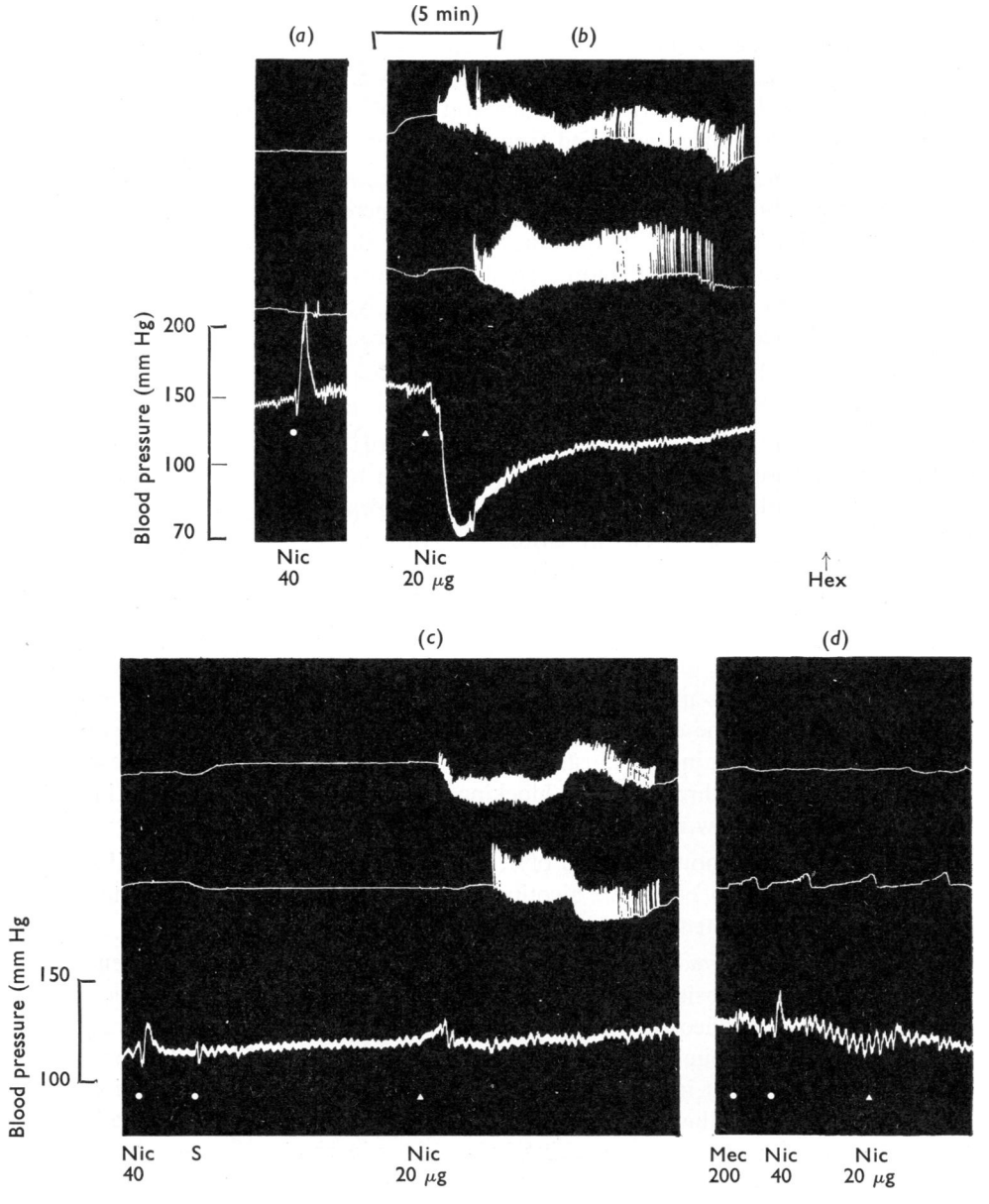


Fig. 2. Cat, 2.7 kg, chloralose anaesthesia. Upper trace: record of right ear twitches. Middle trace: record of left ear twitches. Lower trace: femoral blood pressure. Nic=nicotine, with doses as μg/kg injected intravenously at the white dots. At the white triangles 20 μg of nicotine was injected into the right lateral ventricle. Mec=mecamylamine, the dose expressed as μg/kg injected intravenously. S=2 ml. saline injected intravenously. Between (*b*) and (*c*) two intravenous injections of hexamethonium were given, each of 0.2 mg/kg (Hex). Time intervals: between (*a*) and (*b*), 45 min ; (*b*) and (*c*) 27 min ; (*c*) and (*d*), 25 min.

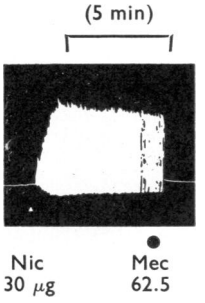


Fig. 3. Cat, 3.2 kg. chloralose anaesthesia. Record of left ear twitches. At the white triangle 30 μg of nicotine was injected into the right lateral ventricle. The ears were still twitching vigorously and frequently when 62.5 μg/kg of mecamylamine was injected intravenously 6½ min later. Ear twitching stopped within 30 sec of the injection of mecamylamine.

first 2 min of its application but in only one experiment was the ear twitching prolonged. In some experiments physostigmine itself and neostigmine caused isolated ear twitching soon after their administration.

Acetylcholine. Injected intraventricularly or applied topically to the cervical cord, acetylcholine in concentrations up to 10^{-3} g/ml. caused ear twitching and facilitation of the pinna reflex in only one experiment. If, however, acetylcholine and physostigmine were injected together on to the cord through the atlanto-occipital membrane, a brief

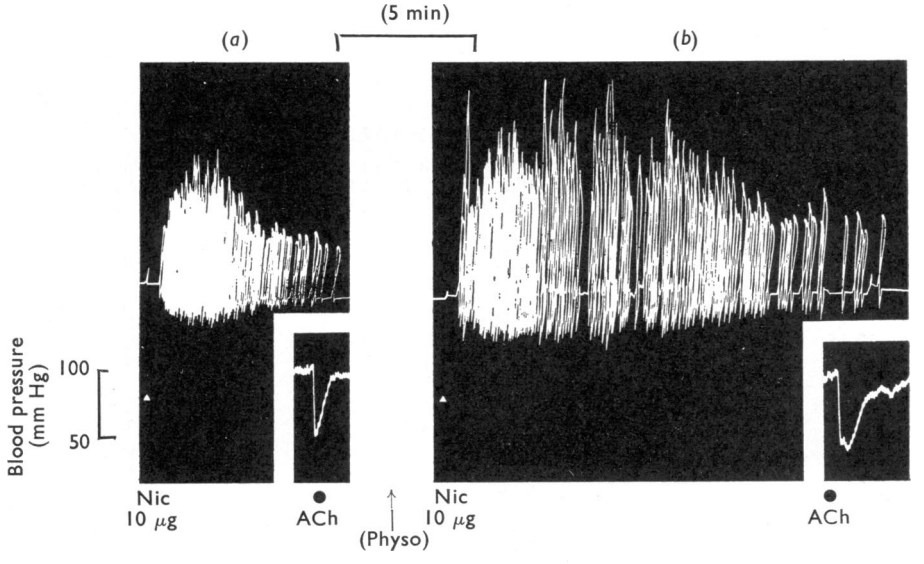


Fig. 4. Cat, 2.9 kg, chloralose anaesthesia. Upper trace: record of right ear twitches. The two lower insets show the effect of 2 μg of acetylcholine (ACh) injected intravenously on femoral blood pressure. At the white triangles, 10 μg of nicotine was injected into the right lateral ventricle. Between (*a*) and (*b*), four intravenous injections of physostigmine were given, each of 0.1 mg (Physo), after which the ear response caused by 10 μg of nicotine was potentiated and also the effect of 2 μg acetylcholine (ACh) on blood pressure. Time interval between (*a*) and (*b*), 28 min.

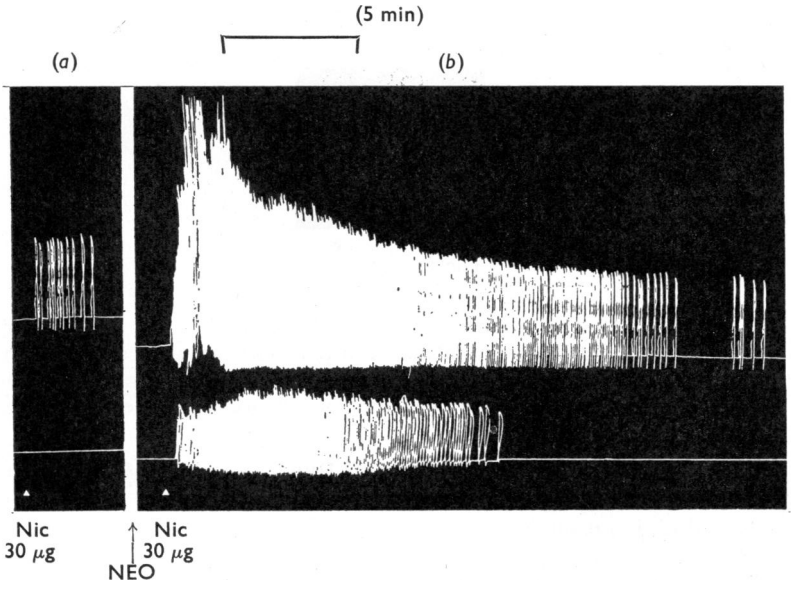


Fig. 5. Cat, 3.2 kg, chloralose anaesthesia. Upper trace: record of left ear twitches. Lower trace: record of right ear twitches. At the white triangles, 30 μg of nicotine was injected into the right lateral ventricle. Between (*a*) and (*b*) 20 μg of neostigmine was injected intraventricularly (Neo). 30 min later the effect of 30 μg of nicotine injected intraventricularly was greatly potentiated and the right ear twitched for the first time following an intraventricular injection of nicotine. Time interval between (*a*) and (*b*), 86 min.

TABLE 2

CAT, 3·4 KG, CHLORALOSE ANAESTHESIA

20 μg of nicotine was injected into the right lateral ventricle at approximately hourly intervals. The data in the table show that, after a single intraventricular injection of 20 μg of neostigmine, the duration of nicotine-induced ear twitching was greatly increased

Time	Ear (L = left; R = right)	Duration of ear twitching (sec)
11.15	L	198
	R	303
12.15	L	390
	R	339
12.52	Neostigmine	
1.12	L	1,152
	R	1,073
2.22	L	840
	R	921
3.18	L	251
	R	329

burst of ear twitching regularly resulted. This is illustrated in Fig. 6. In this experiment physostigmine facilitated the pinna reflex for a few minutes after its application.

Nicotine-like compounds. DMPP and nicotine monomethiodide when administered intravenously in amounts up to 1 mg/kg, did not cause ear twitching. On the other hand, anabasine, nornicotine and metanicotine caused ear twitching when given intravenously in amounts of 0.5 mg/kg (Fig. 7). With metanicotine the response consisted

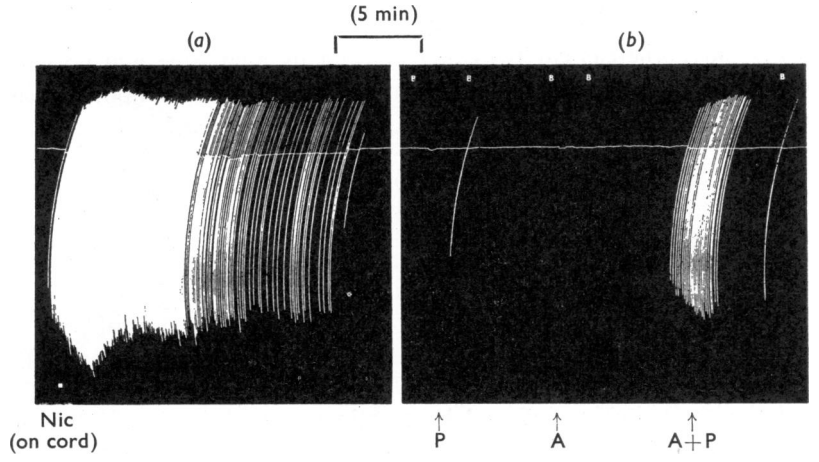


Fig. 6. Cat, 3 kg, chloralose anaesthesia. Record of right ear twitches. At the white square, 0.2 ml. of a solution containing 10^{-4} g/ml. of nicotine was injected on to the upper cervical cord through the atlanto-occipital membrane (*a*). In (*b*) effect of 0.2 ml. of a solution containing 10^{-5} g/ml. of physostigmine (P), 0.2 ml. of a solution containing 10^{-4} g/ml. of acetylcholine (A) and 0.2 ml. of a solution containing 10^{-4} of acetylcholine and 10^{-5} g/ml. of physostigmine (A+P). All 3 solutions injected in the same way as the nicotine. At B the right ear was gently blown. Time interval between (*a*) & (*b*), 20 min.

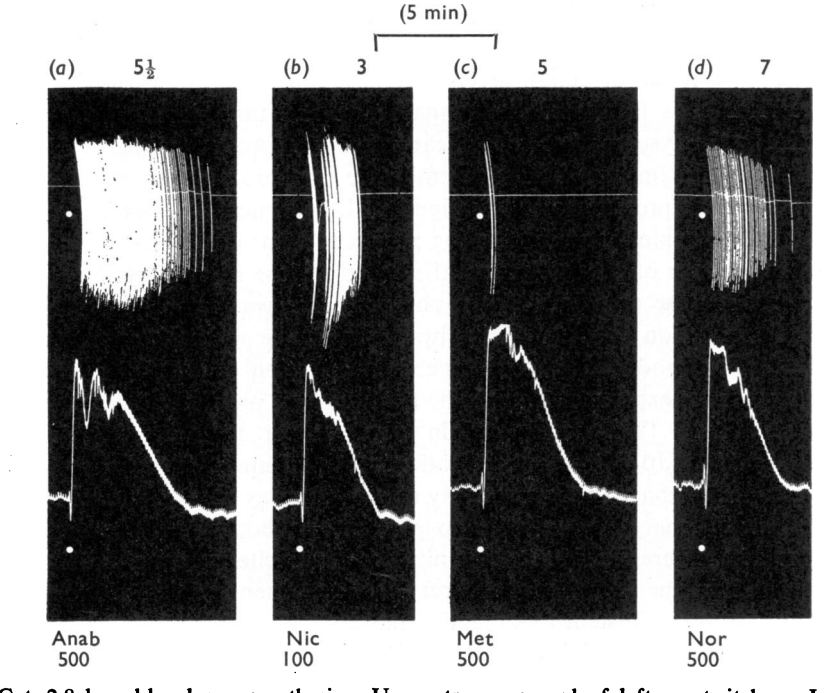


Fig. 7. Cat, 2.8 kg, chloralose anaesthesia. Upper trace: record of left ear twitches. Lower trace: record of femoral blood pressure. Anab=anabasine, Nic=nicotine, Met=metanicotine, Nor=nornicotine, with doses as μg/kg injected intravenously. During the break in the ear record in (*b*), the ears were folded back. The figures at the top of each section give duration of right ear response in min. Time intervals: between (*a*) and (*b*), 8 min ; (*b*) and (*c*), 9 min ; (*c*) and (*d*), 22 min.

of two twitches only, whereas ear twitching induced by anabasine and nornicotine was comparable to that caused by 100 μg nicotine/kg. All these compounds caused ear twitching also when applied topically to the exposed cervical cord.

DISCUSSION

An important finding of the present experiments was the observation that under the conditions described a central action of nicotine was potentiated by physostigmine and neostigmine. It is well known that the entry of drugs into the central nervous system depends on their lipid solubility (Brodie & Hogben, 1957). For this reason, quaternary substances do not readily penetrate the central nervous system, and the fact that neostigmine affected the ear twitching induced by nicotine only when given intraventricularly but not when given intravenously suggested that the potentiating effect of neostigmine, and presumably physostigmine as well, was one within the central nervous system. It therefore seems likely that the potentiation of the ear twitching produced by neostigmine and physostigmine is due to inhibition of brain cholinesterase. The possibility of another mode of action, however, cannot be fully excluded since anticholinesterases have pharmacological actions not attributable to inhibition of cholinesterase (Holmstedt, 1965).

It is possible that nicotine causes twitching of the ears by mimicking the action of, or perhaps releasing acetylcholine in the central nervous system. Knapp & Domino (1962) have suggested that nicotine acts in this way on the ascending reticular activating system. In favour of such a mode of action are the observations that physostigmine applied to the upper cervical cord facilitated the pinna reflex and that simultaneous application of acetylcholine and physostigmine caused ear twitching. Acetylcholine applied by itself was ineffective. These findings bear a resemblance to those of Bülbring & Burn (1941) who found that, in the presence of physostigmine, 1 μg of acetylcholine injected into the artery of the perfused spinal cord of the dog produced contraction of the tibialis anterior muscle. In the absence of physostigmine, the effective dose of acetylcholine varied from 60 μg to 1 mg. In these experiments, the physostigmine was almost certainly acting by preventing the destruction of acetylcholine by cholinesterase. An alternative explanation for the ear twitching produced by simultaneous application of acetylcholine and physostigmine in the present experiments might be the ability of physostigmine to affect acetylcholine permeability. This would explain why the ear twitching began within the relatively short time of 10–15 sec of application. The failure to demonstrate that acetylcholine alone did not behave in the same way as nicotine may merely have been due to the inability of the quaternary acetylcholine to penetrate without physostigmine to the site where nicotine acts. There is evidence that nicotine can excite neurones in the spinal cord and the thalamus in the same way as acetylcholine, when applied electrophoretically (Curtis, 1965). In the brainstem too, nicotine has an excitant action on neurones excited by acetylcholine (Bradley & Wolstencroft, 1965).

The observations made with the secondary, tertiary and quaternary ganglion-blocking drugs (mecamylamine, pempidine and hexamethonium) and tertiary and quaternary cholinesterase inhibitors (physostigmine and neostigmine) provide convincing pharmacological evidence that the ear twitching induced by nicotine is of central origin. Given intravenously, only mecamylamine and pempidine antagonized, and only physostigmine

potentiated this action of nicotine. Given intraventricularly, the quaternary were as effective as the secondary and tertiary compounds. The fact that DMPP and nicotine monomethiodide did not cause ear twitching when injected intravenously, although they did when injected intraventricularly, was further evidence that the ear twitching response was mediated centrally.

Substances injected intraventricularly may act from the inside of the brain on structures reached from the cerebral ventricles, or they may act from the outside of the brain on structures reached from the subarachnoid space (Feldberg, 1963). Possible sites of action of nicotine were therefore numerous. The finding that the ear response to intravenous nicotine was obtained in decerebrate cats and after transection of the cord at C2, but abolished after transection at the level of C1, suggests that nicotine is acting on the upper cervical cord. Applied topically to this site, nicotine produced the typical ear response and this site of action is in agreement with the findings and conclusions of Hall & Reit (1965). Whether the action of nicotine is on structures within the cord itself is not certain.

When nicotine was injected into a lateral ventricle, it therefore had to reach the cervical cord before twitching of the ears occurred. Access to the cord was via the foramina of Luschka in either side of the fourth ventricle. It would be expected that the nicotine would flow down both sides of the cord and thus cause both ears to twitch. This frequently occurred, as did twitching of the ear on the same side as the intraventricular injection was made. In a minority of experiments, however, only the left ear twitched following an injection of nicotine into the right lateral ventricle.

It is interesting that the region of the spinal cord on which nicotine caused twitching of the ears and facilitation of the pinna reflex was almost identical with the region on which bromophenol blue and tubocurarine caused scratching movements and facilitation of the scratch reflex (Feldberg & Fleischhauer, 1960 ; Domer & Feldberg, 1960 ; Feldberg, 1962). Scratching movements were observed under pentobarbitone sodium but not under chloralose anaesthesia, whereas the ear twitching occurred under either anaesthetic.

The efficiency of mecamylamine and pempidine administered intravenously in blocking the nicotine-induced ear twitching is another example of a central action of nicotine that is readily blocked by secondary and tertiary but not quaternary ganglion-blocking drugs. Knapp & Domino (1962) showed that mecamylamine, but not hexamethonium, blocked nicotine induced EEG activation. Domino (1965) showed that mecamylamine was more effective than the quaternary compound trimethidinium in preventing the depression of a conditioned avoidance reflex in rats by nicotine. In the present experiments large doses of hexamethonium, given intravenously, did block the ear twitching induced by nicotine but only after a time lapse of at least one hour. Although cerebrospinal fluid levels of hexamethonium may be less than 1% of the plasma level, even after 1–2 hr (Paton, 1958) this concentration of hexamethonium could apparently be sufficient to reduce or abolish the nicotine induced ear twitching.

SUMMARY

1. Nicotine was injected directly into the cerebral ventricles of conscious and anaesthetized cats. A striking and regular effect of such injections was a rapid twitching

of the ears, starting within 20 sec of the injection and continuing for up to 30 min. In some cats as little as 1.25 μg of nicotine base was sufficient to elicit this response. Other effects observed with amounts up to 100 μg were salivation, licking, retching, vomiting, violent head shaking and panting. On intravenous injection, nicotine had to be given in amounts of at least 100–200 μg to cause twitching of the ears. The typical ear response was also elicited by topical application of nicotine in a concentration from 10^{-5} to 10^{-4} g/ml. to the upper cervical cord.

2. The quaternary ganglion-blocking drug hexamethonium effectively prevented or abolished the nicotine induced ear twitching when administered intraventricularly but not when administered intravenously. The secondary and tertiary compounds mecamylamine and pempidine, on the other hand, antagonized this action of nicotine when given by either route.

3. The tertiary anticholinesterase physostigmine potentiated the nicotine-induced ear twitching when injected intravenously or intraventricularly. The quaternary anticholinesterase neostigmine, however, potentiated the ear response only when injected intraventricularly.

4. The observations made with the different ganglion-blocking drugs and cholinesterase inhibitors suggested that the ear twitching induced by nicotine is of central origin and observations of the effects of spinal section showed that the site of action of nicotine is localized to the upper cervical cord.

5. Acetylcholine applied to the cord simultaneously with physostigmine caused a short burst of ear twitching. The possibility is discussed that nicotine acts by mimicking the action of, or releasing, acetylcholine. The property of causing twitching of the ears is not specific to nicotine but is shared by other nicotine-like compounds. None of the compounds which were tested, however, was as effective as nicotine. Quaternary compounds like DMPP and nicotine monomethiodide caused twitching of the ears when injected intraventricularly but not when injected intravenously.

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