THE PHARMACOLOGY OF NICOTINE MONOMETHIODIDE

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INTRODUCTION

NICOTINE is a ditertiary base. It forms two monomethiodides and a dimethiodide (Fig. 1). Nicotine is of great interest because, although it does not contain an "-onium" group, it both stimulates and then blocks the ganglionic and neuromuscular synapses. Nicotine dimethiodide (NDM) was shown by Crum-Brown and Fraser in 1869[1] to paralyse a frog nerve-muscle preparation in a curare-like manner and to be much less toxic than the parent alkaloid, nicotine. Nicotine dimethiodide has two quaternised nitrogen atoms and neuromuscular blocking activity might not be unexpected.
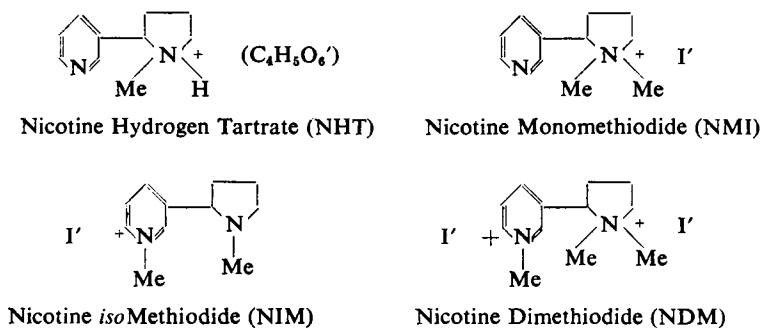

Nicotine Hydrogen Tartrate (NHT) Nicotine Monomethiodide (NMI)

Nicotine *iso*Methiodide (NIM) Nicotine Dimethiodide (NDM)

FIG. 1.

Nicotine *iso*methiodide (NIM) and NDM in 5 mg. doses were shown by Burn[2] not to raise the blood pressure of the spinal cat. Nicotine monomethiodide (NMI), a crystalline, but extremely hygroscopic substance, which contains one molecule of water of crystallisation, was prepared by Barlow and Dobson in 1955[3]. Barlow and Dobson[3] also made a preliminary study of the pharmacological properties of this compound. In two experiments on the blood pressure of the spinal cat, they found that NMI was roughly as active as nicotine hydrogen tartrate (NHT), although in one other experiment, it was found to have only 40 per cent. of the pressor activity of the latter. Barlow and Dobson[3] also pointed out that the pressor response to NMI differed in form from that to NHT.

Some degree of sensitisation to the monomethiodide was shown. 140 mg. of NMI in doses of 10 mg. given over a period of one hour, did not produce complete ganglion block but the pressor effect of a subsequent dose of 1 mg. of NHT was reduced. Prior injection of 0·25 mg. of hexamethonium bromide almost abolished the pressor effects of 0·6 mg.

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NHT and of 0·6 mg. NMI. Taylor[4] has calculated from the dissociation constants of nicotine, that it exists at body pH, as the univalent nicotinium ion. In this form, the cationic head—N^+MeH—is presented to the receptor surface by the pyrrolidine ring. Barlow[5] points out that if the active species is the nicotinium ion then the corresponding N-methyl quaternary salt (NMI) would be expected to be much more potent than nicotine itself. When the pyridine nitrogen is quaternised as in NIM, the activity should be much reduced.

This account deals with some aspects of the pharmacology of NMI, NDM and NIM supplied by Drs. Barlow and Dobson.

All drug concentrations refer to final bath concentrations.

EXPERIMENTAL METHODS AND RESULTS

Guinea-pig Terminal Ileum. A strip of guinea-pig terminal ileum, about 2 cm. long, was suspended in a 2 ml. bath containing oxygenated Tyrode's solution at 33° C. Nicotine monomethiodide stimulated the gut at doses of 5 to 500 μg./ml. without producing tachyphylaxis. At the beginning the response to NMI was small, but as the experiment proceeded and other active drugs (histamine and acetylcholine) were used, the NMI response increased to a constant level after about an hour. This response to a given dose was maintained throughout the rest of the experiment. Tested over a dose range of 25 to 500 μg./ml. NHT and NMI appeared to be equipotent. The contractions induced by 100 μg./ml. NMI were completely inhibited by 50 μg./ml. papaverine sulphate (Fig. 2), which on the same preparation inhibited the responses to 25 mg./ml. of barium chloride and 1 μg./ml. of acetylcholine bromide. It was much more difficult
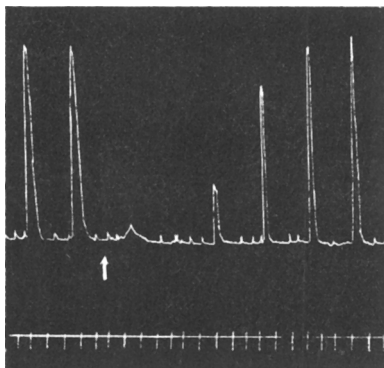

FIG. 2. Inhibitory effect of papaverine on NMI contractions of the guinea-pig terminal ileum. 100 μg./ml. NMI added every 2 minutes. At arrow, 50 μg./ml. of papaverine sulphate was added 1 minute before NMI. Time signal = 1 minute.

to obtain constant, reproducible responses on the ileum to NHT than to NMI. 0·25 μg./ml. atropine sulphate inhibited the response to 250 μg./ml. NMI (Fig. 3). 250 μg./ml. of hexamethonium bromide considerably reduced but did not completely eliminate the response to 100 μg./ml. NMI (Fig. 4). 25 μg./ml. to 1 mg./ml. NIM and 50 μg./ml. to 1 mg./ml. NDM did not stimulate the ileum.

Virgin Rat Uterus. Œstrus was induced in rats weighing between 185 and 220 g. by means of a subcutaneous injection of 10 μg./100 g. body weight of stilbœstrol in arachis oil, 24 hours before use. One horn of the uterus was suspended in a 2 ml. bath containing oxygenated de Jalon's solution at 29° C. No direct action was seen with doses of NMI

from 25 to 500 μg./ml., NDM from 500 μg. to 1 mg./ml., NIM from 500 μg. to 1 mg./ml., or NHT from 100 μg. to 1 mg./ml.

Frog Sartorius Muscle—Ischiad Nerve Preparation. This preparation was used in a 50 ml. bath containing oxygenated Frog Ringer's solution at room temperature. The nerve was stimulated by means of a square
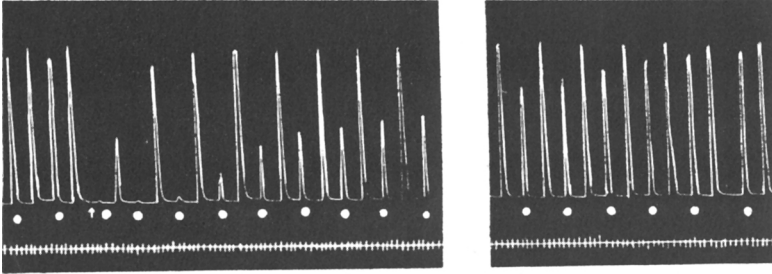

FIG. 3. Inhibitory effect of atropine on NMI contractions of the guinea-pig terminal ileum. At dots 250 μg./ml. NMI added. Unmarked responses due to 0·25 μg./ml. of acetylcholine bromide. At arrow, 0·25 μg./ml. of atropine sulphate added 1 minute before NMI. Time signal = 30 seconds.

wave stimulator delivering 7 stimuli per minute. The duration and voltage of the impulse were 1·1 millisecs. and 40 volts respectively. 5 μg./ml. NMI produced a slight potentiation of the response with no inhibition. 10 μg./ml. NMI caused slight inhibition, followed by slight potentiation of the response. 40 μg./ml. NMI caused spontaneous twitching and a fall in tone. At this dose level there was complete inhibition of the contractions (Fig. 5), which was reversible on washing.
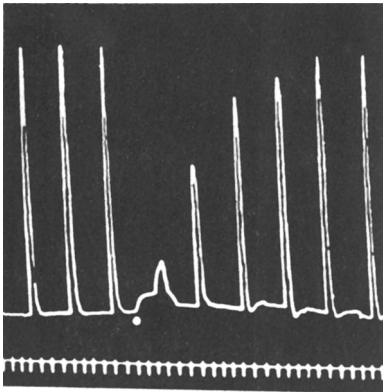
Rat Phrenic Nerve—Diaphragm Preparation. This preparation was suspended in a 100 ml. bath containing oxygenated Tyrode's solution with double the normal amount of glucose. The muscle was stimulated indirectly by the nerve and also directly by means of a square wave stimulator delivering 7 stimuli per minute. The duration and voltage of the impulse were 1·1 millisecs. and 40 volts respectively. 5 to 10 μg./ml. of NMI produced neither potentiation nor inhibition of the response to indirect stimulation of the muscle. Doses of from 20 to 60 μg./ml. NMI gradually inhibited the response. There was a roughly graded relation between the dose and the degree of inhibition. When indirect
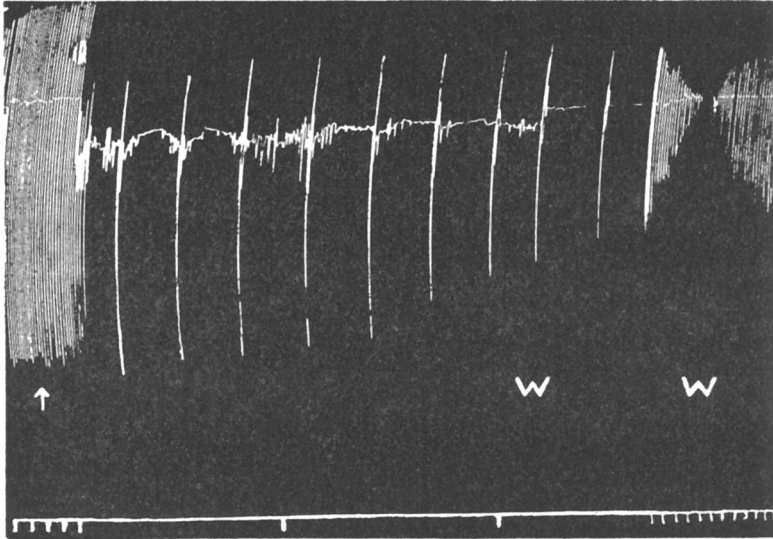

FIG. 4. Inhibitory effect of hexamethonium on NMI contractions of the guinea-pig terminal ileum. 100 μg./ml. NMI added at 2-minute intervals. At dot, 250 μg./ml. hexamethonium bromide added 1 minute before NMI. Time signal = 1 minute.

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FIG. 5. Effect of NMI on neuromuscular conduction in the frog sartorius —ischiad preparation. At arrow, 40 μg./ml. NMI added. At W, bath emptied and refilled with fresh frog ringer's solution. Time signal = 1 minute.

stimulation failed to produce a response, direct stimulation of the muscle caused a small contraction (Fig. 6). NMI appeared to be about twice as potent as NHT in inhibiting the electrically induced contractions of the rat diaphragm. Doses of 50 to 250 μg./ml. NDM and NIM had no observable effect on this preparation.

Frog Rectus Abdominis Muscle. The frog rectus abdominis muscle strip was suspended in a 10 ml. bath containing oxygenated Frog Ringer's solution at room temperature. NMI had a stimulant action on the rectus and gave graded and reproducible responses to doses of from 0·05μg. to 2·5 μg./ml. There was no twitching and no sign of tachyphylaxis. On this preparation, NHT was always found to be slightly less potent than NMI. NDM at doses of from 50 μg. to 500 μg./ml. had a stimulant action and produced graded and reproducible
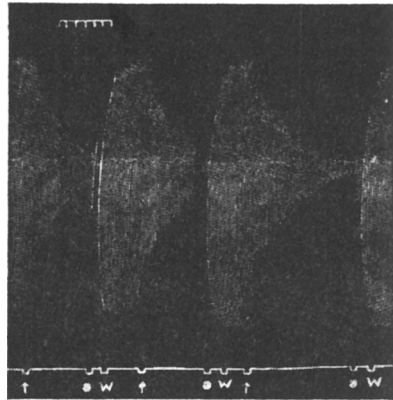

FIG. 6. Effect of NMI on neuromuscular conduction in the rat phrenic nerve-diaphragm preparation. At arrow, 50 μg./ml. NMI added to bath. At W, bath emptied and refilled with fresh, double glucose, Tyrode's solution. At dot, direct, electrical stimulation of diaphragm. Time signal = 1 minute.

responses between those doses. 0·5 mg. to 1·0 mg./ml. NIM produced no response on this preparation.

Perfused Frog's Heart. Perfusion was made through the *sinus venosus* with oxygenated Frog Ringer's solution at room temperature. Drugs were administered in solution, by injection into the side arm of the perfusion cannula. Doses of from 0·5 μg. to 1 mg. NMI in 1 ml. had no noticeable effects, but 2 mg. caused an increase in amplitude and 5 mg. a marked increase in amplitude with a slight slowing of the heart.

Blood Pressure and Respiration of the Chloralosed Cat. Anæsthesia was induced in cats weighing between 2·75 and 3·5 kg. with ether or an ether-chloroform mixture and maintained with chloralose. Drugs were administered into a cannula inserted into the external jugular vein and arterial blood pressure was recorded from the carotid artery in the usual way. To record respiration, a thread was sewn into the abdominal wall and led over pulleys to a suitable recording lever. Cats varied in their response to NMI. A dose of NMI which in one cat would produce a large rise in b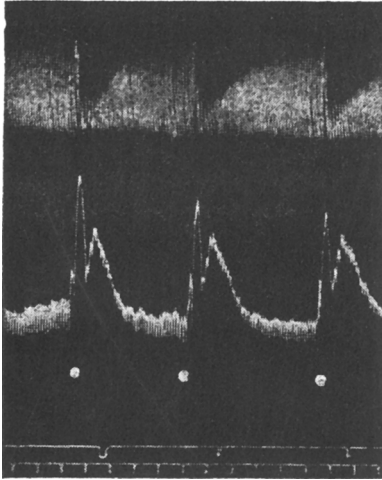lood pressure, with marked muscle twitching, respiratory depression and a short period of apnœa would, in another, cause only a slight pressor effect with a momentary depression of respiration. NMI appeared to have a cumulative effect on respiration, and after a series of doses it was usually necessary to give artificial respiration. In the majority of cats, spontaneous respiration appeared to be irreversibly inhibited and artificial respiration had to be given for the remainder of the experiment. The characteristic effects of 0·5 mg. of NMI in a 3 kg. cat were: very slight stimulation followed by marked depression of respiration with apnœa. If spontaneous respiration recovered, the depth was usually markedly increased. We did not observe the marked respiratory stimulation which followed NHT. The effects on blood pressure of doses of NMI of from 0·1 to 1·0 mg. appeared to be graded and often showed a secondary, more prolonged rise (Fig. 7). 0·25 mg. ergotoxine ethanosulphonate reduced the height of the response to NMI and where there was a secondary rise, this was inhibited. 0·15 mg. Hydergine reversed the pressor effects of 0·4 mg. NMI. 1 mg. of hexamethonium bromide almost abolished the pressor response to a similar dose of NMI. 1 mg. tetra-ethylammonium bromide reduced, but did not abolish the pressor response to 0·2 mg. NMI (Fig. 8) and the pressor effects of 1·5 mg. NMI were partly blocked

FIG. 7. Chloralosed cat: upper record, respiration, lower record, blood pressure. At dot, 0·25 mg. NMI + 3 ml. saline added. Time signal = 30 seconds.
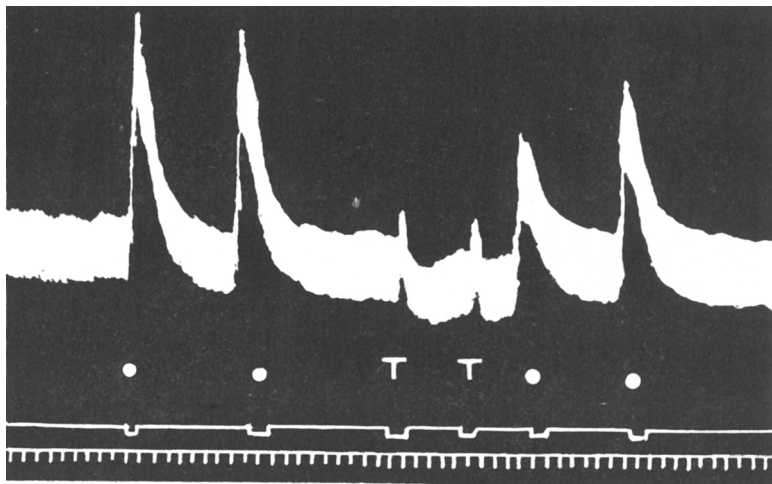
FIG. 8. Inhibitory effect of TEA on NMI pressor response. Chloralosed cat: blood pressures. At dots, 0·2 mg. NMI + 3 ml. saline added. At T, 1·0 mg. tetra-ethyl ammonium bromide + 3 ml. saline added. Time signal = 30 seconds.

by 1 mg. atropine sulphate (Fig. 9). In one cat only, a 1 mg. dose of NMI produced a diphasic depressor-pressor response. NMI appeared to have from 40 to 80 per cent. of the pressor activity of NHT in this series of experiments. It was more difficult to produce tachyphylaxis to
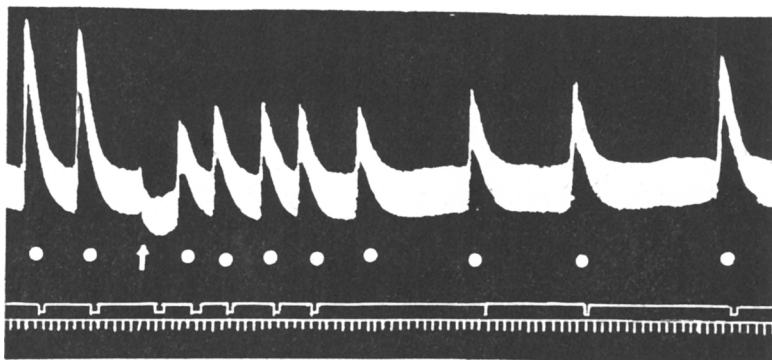

FIG. 9. Effect of Atropine on NMI pressor response. Chloralosed cat: blood pressure. At dots, 1·5 mg. NMI + 3 ml. saline added, at arrow, 1·0 mg. atropine sulphate + 3 ml. saline. Time signal = 30 seconds.

NMI on this preparation than to NHT. In one preparation, for example, tachyphylaxis was produced by giving eight 0·5 mg. doses of NHT over a period of 5 minutes. On the same preparation, however, fourteen 0·5 mg. doses of NMI had to be given in 5 minutes to produce a similar effect. 25 mg. NDM produced a slight depressor response and 25 mg. NIM produced a diphasic depressor-slight pressor response.

Blood Pressure of the Spinal Cat. Once again the magnitude of the pressor response varied from cat to cat. The pressor response to 1 mg. NMI could be completely blocked by 1 mg. of hexamethonium bromide (Fig. 10). NMI appeared to have about 40 per cent. of the potency of
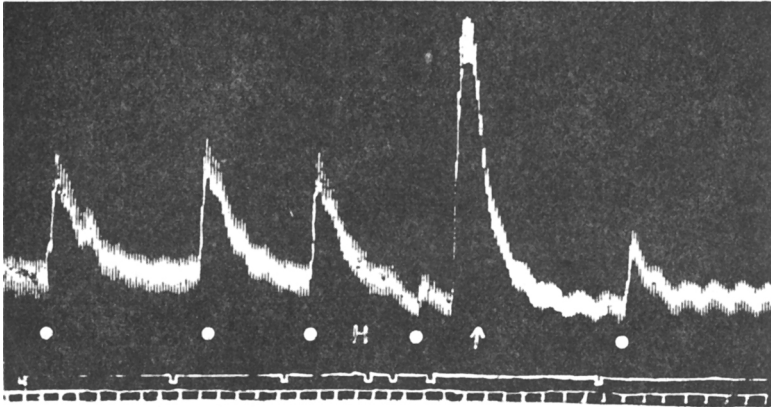

FIG. 10. Inhibitory effect of hexamethonium on NMI pressor response. Spinal cat: blood pressure. At dot, 1 mg. NMI + 3 ml. saline added; at H, 1 mg. hexamethonium bromide in 3 ml. saline added, at arrow; 5 μg. adrenaline + 3 ml. saline added. Time signal = 1 minute.

NHT on this preparation (Fig. 11). It was difficult to produce ganglion block. In one instance, 70 mg. NMI in 5·0 mg. doses was effective when given in the space of 3·5 minutes (Fig. 12). Recovery was rapid. 10 mg. of NDM and 5 mg. of NIM had no effect on the blood pressure of the spinal cat.
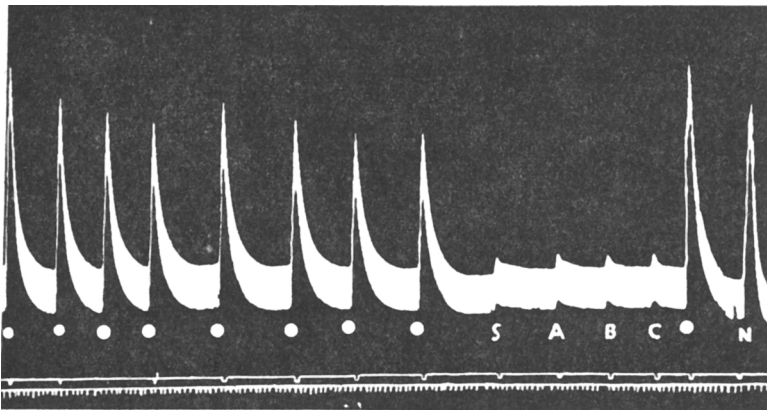

FIG. 11. Spinal cat: blood pressure. At dots, 5 mg. NMI + 3 ml. saline added; at S, 3 ml. saline added; at A, 1 mg. NDM + 3 ml. saline added; at B, 3 mg. NDM + 3 ml. saline added; at C, 5 mg. NDM + 3 ml. saline added; at N, 2 mg. NHT + 3 ml. saline added. Time signal = 30 seconds.

Nictitating Membrane of the Chloralosed Cat. 1 mg. of NMI produced a contraction of the nictitating membrane. In one preparation, when 30 mg. NMI were given over a period of 4 minutes in 5 mg. doses, stimulation and then block of the superior cervical ganglion was produced.
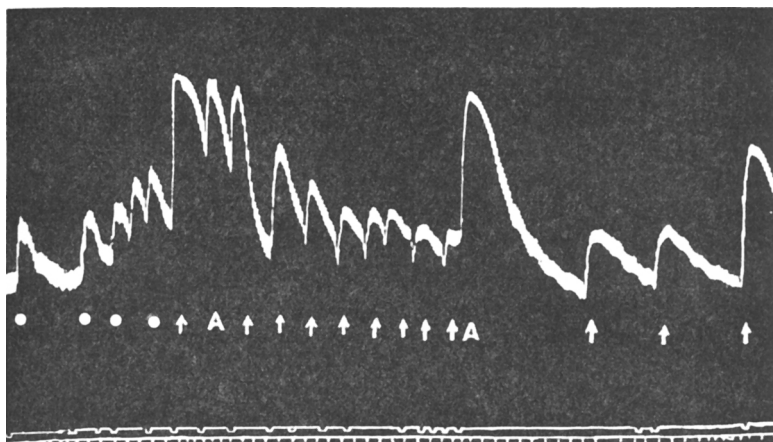

FIG. 12. Ganglion block produced by NMI. Spinal cat: blood pressure. At dots, 1 mg. NMI + 3 ml. saline added; at arrows, 5 mg. NMI + 3 ml. saline added; at A, 10 μg. adrenaline + 3 ml. saline added. Time signal = 30 seconds.

CONCLUSIONS

On skeletal muscle NMI appears to be somewhat more potent than NHT. It has a slightly greater stimulant action on the frog rectus and depresses the rat diaphragm more effectively than NHT at similar doses. NMI is a more potent respiratory depressant than NHT and seems to have a cumulative effect on respiration. NMI is less active in both stimulating and depressing the autonomic ganglia which influence the blood pressure. Although it may be more firmly held on to the receptor surfaces[5], the nicotinic activity of NMI does not appear to be increased, at any rate at the ganglionic synapse. In stimulating frog skeletal muscle, there is little difference in the potency of the two compounds; on the other hand NMI is much more effective in depressing the rat diaphragm. These differences may be a reflection of varying degrees of accessibility of the receptors at these different sites to the quaternary compound, or there may be differences in the susceptibility to enzymic attack. It is worth speculating whether the normal metabolism of nicotine includes a quaternary compound, at an early stage. This would be an analogue of the monomethiodide. Possibly one of the hydrogen atoms on the pyrrolidine nitrogen is replaced by another group. In NMI a methyl group has replaced this hydrogen atom. This may make it more susceptible to further attack. The next step might be the formation of a compound analogous to the *iso*methiodide which we have found to be completely devoid of nicotinic properties. The dimethiodide still possesses slight nicotinic activity on the blood pressure of the chloralosed cat and on the

frog rectus abdominis, but it is very much less active than either the hydrogen tartrate or the monomethiodide.

The inhibitor action of atropine towards the NMI-induced contractions of the guinea-pig ileum (Fig. 3), and the NMI-induced pressor response of the chloralosed cat (Fig. 7) is interesting. This effect was not seen with NHT.

SUMMARY

1. Nicotine monomethiodide had a direct stimulant action on guinea-pig ileum and frog rectus abdominis muscle which with ileum was antagonised by atropine and hexamethonium.

2. The pressor response to nicotine monomethiodide of the chloralosed and spinal cat was qualitatively similar to that of nicotine hydrogen tartrate and could be modified or abolished by atropine, tetra-ethyl ammonium or hexamethonium.

3. The effect of nicotine monomethiodide on respiration appeared to be cumulative and was characterised by slight initial stimulation followed by depression and then apnœa. This effect differed from that of nicotine hydrogen tartrate.

4. Nicotine monomethiodide was more potent in depressing the rat phrenic nerve diaphragm preparation than nicotine hydrogen tartrate and in very large doses increased the amplitude of the perfused frog heart.

5. The possible significance of the varying nicotinic activity in the series nicotine hydrogen tartrate, nicotine monomethiodide, nicotine dimethiodide and nicotine isomethiodide is discussed.

REFERENCES

1. Crum-Brown and Fraser, Trans. roy. Soc., Edin., 1869, 25, 151.
2. Burn, Quoted by Barlow and Dobson, J. Pharm. Pharmacol., 1955, 7, 27.
3. Barlow and Dobson, ibid., 1955, 7, 27.
4. Taylor, Pharmacol. Rev., 1951, 3, 412.
5. Barlow, Introduction to Chemical Pharmacology, Methuen and Co., Ltd., London, 1955.