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Antinociceptive action of nicotine and its methiodide derivatives in mice and rats

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1 Three quaternary methiodides of nicotine were prepared and tested for antinociceptive activity in the mouse tail-flick, mouse phenylquinone and rat tail-flick tests.

2 Following peripheral administration, all three methiodides were inactive in the mouse and rat tail-flick procedures, whereas nicotine was active in both tests, which suggested that nicotine was acting centrally.

3 Quaternization of nicotine did not eliminate antinociceptive activity as demonstrated by the intraventricular injection of the methiodides in mice. Nicotine pyrrolidine and *bis* methiodides were somewhat more potent than nicotine, whereas nicotine pyridine methiodide was considerably less potent than nicotine in the tail-flick procedure.

4 Systemically administered nicotine pyrrolidine methiodine was approximately one-third as active as nicotine in the mouse phenylquinone test; nicotine pyridine methiodide and nicotine *bis* methiodide were 100 and 300 times less active, respectively.

5 Hexamethonium partially blocked nicotine and nicotine pyrrolidine methiodide, whereas mecamylamine blocked nicotine completely but nicotine pyrrolidine methiodide partially. Nicotine may have both central and peripheral actions in the mouse phenylquinone test, whereas nicotine pyrrolidine methiodide may have both nicotine and non-nicotine like antinociceptive activity.

6 The radiolabelled methiodides were synthesized and their disposition in body tissues studied. The methiodides were found to penetrate brain poorly (plasma-to-brain ratios > 20).

7 The methiodides were metabolized to nicotine to a small extent. This metabolism occurred to a greater extent in mice than in rats.

Introduction

Nicotine produces antinociception in mice and rats (Mansner, 1972; Sahley & Bernston, 1979; Aceto, Martin, Tripathi, May & Jacobson, 1980; Tripathi, Martin & Aceto, 1982; Phan, Doda, Bile & Gyorgy, 1983) comparable in potency to that of morphine (Dewey, Harris, Howes & Nuite, 1970). However the question whether the action of nicotine is central and/or peripheral has not been resolved.

The methiodides of nicotine have been used extensively as probes for distinguishing the central and peripheral actions of nicotine (Domino, 1965; Geller, Hartman & Blum, 1971; Schechter & Rosecrans, 1972; Thompson, Angulo, Choi, Roch & Jenden, 1972). The loss of central activity after quaternization of nicotine could be due to a decrease in the penetrability of the blood-brain barrier or to an alteration in the pharmacological activity or a combination of both. The peripheral actions of the methiodides have been studied quite extensively. Gillis & Lewis (1956) showed that nicotine pyrrolidine methiodide had potent nicotine-like properties, nicotine *bis* methiodide possessed slight nicotine activity and nicotine pyridine methiodide was devoid of nicotine-like effects when assayed on quinea-pig ileum, frog rectus abdominis muscle and rat phrenic diaphragm preparations and for pressor effects in cats. Barlow & Dobson (1955) had reported earlier that nicotine pyrrolidine methiodide and nicotine were equipotent in their pressor effects in cats, whereas the two other methiodides were at least 10 times less active. These results were in agreement with the report of Larson & Haig (1943) that nicotine and nicotine pyrrolidine methiodide were equally toxic in mice and rats and both produced pressor effects in dogs to a similar degree. It is clear from the above mentioned studies that quaternizing nicotine

may alter its pharmacological activity. Therefore, our approach has been to attempt to establish a centrally active dose of the quaternaries when they are given peripherally, rather than merely study their central effect in dose ranges in which nicotine is active, as other investigators have done (Domino, 1965; Geller, *et al.*, 1971; Schechter & Rosecrans, 1972).

We decided to synthesize and test the methiodides of nicotine for antinociceptive activity. In addition, radiolabelled methiodides were prepared so that the degree to which they penetrate the central nervous system could be established as well as the extent to which they are metabolized to nicotine. By studying their disposition and correlating these effects with antinociceptive activity we hoped to resolve the question of the site(s) of action of nicotine.

Methods

Syntheses

Methiodides We have prepared the three possible (−)-nicotine methiodides (Figure 1); (−)-nicotine pyrrolidine methiodide, (−)-nicotine pyridine methiodide, and (−)-nicotine *bis* methiodide essentially by the method of Barlow & Dobson (1955) and Barlow & Hamilton (1962). All were analyzed for C, H, N and I and values found agreed with theory within the limits of error. They were also characterized by n.m.r. and i.r. measurements (which are consistent with the structures shown) and by thin-layer chromatography (t.l.c.). To 5.0 g of nicotine in 25 ml of methanol was added 1.0 ml (2.2 g) of methyl iodide. After 24 h at room temperature, the mixture was evaporated to dryness. Excess nicotine was extracted with two 15 ml portions of anhydrous ether (cooling each time before decanting). The residue was dissolved in about 15 ml of acetone to give (after standing overnight at room temperature) 1.6 g of methiodide mixture, m.p. 90–150°C. This was digested with 50 ml of boiling acetone and the mixture cooled to room temperature. Filtration and washing with acetone gave 0.1 g of nicotine *bis* methiodide, m.p. 217–221°C, R_F 0.05 (Silica-Gel GF, Analtech Uniplates, *n*-butanol/pyridine/acetic acid and H_2O, 4:1:1:1, development time about 1 h, I_2 for colour development). Concentration of the above acetone filtrate and washings to 20–25 ml, seeding and cooling overnight at 20–25°C gave 0.85 g of nicotine pyrrolidine methiodide, m.p. 135–137°C, R_F 0.24 (same system as just described). The ether washings above (30 ml) were treated with 1.0 ml of methyl iodide to give, after 5 days, 2.5 g of fluffy needles, m.p. 140–145°C which according to t.l.c. was at least 90% nicotine pyridine methiodide (R_F 0.08). It was digested with 80–90 ml of boiling acetone, cooled briefly in ice and filtered from nicotine *bis* methiodide. Concentrations of the acetone filtrate and washings to about 40 ml and cooling overnight at +5° gave 1.8 g of nicotine pyridine methiodide, m.p. 163–164°C. Nicotine *bis* methiodide is best made from nicotine and 2.2 equivalents of methyl iodide in methanol. It can be recrystallized from methanol acetone; m.p. 222–225°C.

Radiolabelled methiodides The above synthesis were carried out on a micro scale with (−)-[^{3}H]-nicotine (5 Ci mmol^{-1}) which had been synthesized by us previously (Vincek, Martin, Aceto & Bowman, 1980). (−)-[^{3}H]-nicotine (1.6 mCi) was added to 15 mg of nicotine (free base) in 0.5 ml of methanol followed by 0.5 equivalents (10 µl) of methyl iodide. The solution was allowed to stand overnight at room temperature which provided a mixture of all three methiodides, the major of which was pyrrolidine methiodide. The sample was applied to two Analtech silica gel GF plates (20 × 20 cm, 1000 micron thickness) and developed once in *n*-butanol/pyridine/acetic acid/H_2O (4:1:1:2). The band on each plate corresponding to nicotine pyrrolidine methiodide was removed and washed with methanol which yielded [^{3}H]-nicotine pyrrolidine methiodide that was more than 96% pure. The above radiolabelled synthesis was repeated with an equivalent of methyl iodide which resulted in a greater yield of nicotine pyridine methiodide and nicotine *bis* methiodide. The sample was applied to 6 silica gel plates (5 × 10 cm, 250 micron thickness) which were developed 3 times in the above-mentioned solvent system. The multiple development gave a better separation of nicotine and the

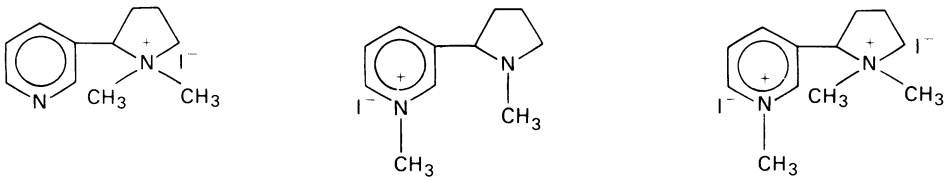

Figure 1 Structures of the nicotine methiodides.

methiodides (nicotine, R_F 0.58; nicotine pyrrolidine methiodide, R_F 0.41; nicotine pyridine methiodide, R_F 0.26; nicotine *bis* methiodide, R_F 0.17). The bands corresponding to nicotine pyridine methiodide and nicotine *bis* methiodide were removed and extracted with methanol which provided pure [^{3}H]-nicotine pyridine methiodide and [^{3}H]-nicotine *bis* methiodide, respectively.

Drug administration

Male Sprague Dawley rats (200–300 g) and Swiss Webster ICR mice (22–30 g) (Dominion Laboratories, Dublin, Va.) were used. All drugs were dissolved in saline and administrated subcutaneously (0.1 ml 10 g^{-1} of body weight for mice and 0.1 ml 100 g^{-1} of body weight for rats) or intraventricularly (5 μl per mouse). The intraventricular (i.c.v.) administration was performed as described by Pedigo, Dewey & Harris (1975). All doses are expressed as μmol kg^{-1} or μmol per animal. The molecular weights for nicotine, nicotine pyrrolidine methiodide, nicotine pyridine methiodide and nicotine *bis* methiodide are 162, 304, 304 and 446, respectively. For both antinociceptive tests 6–12 animals per dose were used. The ED_{50}s and 95% confidence limits were calculated using the method of Litchfield & Wilcoxon (1949). Mecamylamine hydrochloride was a generous gift from Merck (Rahway, N.J.). Hexamethonium bromide was purchased from Sigma Chemical Co. (St Louis, MO.).

Tail-flick test

The procedure used was essentially that of D'Amour & Smith (1941) as modified by Dewey *et al.* (1970). Briefly, the animal's tail was placed in a groove that contained a slit under which was located a photoelectric cell. When the heat source was turned on, it was focused on the tail and the animal responded by flicking its tail out of the groove. As a result, light from the heat source passed through the slit and activated the photocell which stopped a timer. The heat source was calibrated so that the control animals would flick their tails in 2–4 s. A maximal 10 s cut-off latency was imposed if no response occurred. Drug or vehicle was injected 5 min before testing in mice and 2 min before testing in rats. Those times for peak effect were determined for nicotine previously (Tripathi *et al.*, 1982).

Phenylquinone abdominal-stretching test

The procedure of Pearl, Aceto & Harris (1968) was modified as indicated below. Mice were injected with 2.0 mg kg^{-1} i.p. of a para-phenylquinone (PPQ) solution and 5 min later received either nicotine, one of the quarternaries or vehicle. They were then placed in mouse cages in groups of two. Ten min after the PPQ was given, the total number of stretches per group was counted within a 1 min period. The count was repeated 5 min later. A stretch was characterized by an elongation of the mouse's body, development of tension in the muscles in the abdominal region, and an extension of the limbs. The antinociceptive response was expressed as the % inhibition of the stretching response. For antagonism studies, the mice were treated as described above except they received either saline, mecamylamine or hexamethonium 10 min before the PPQ. Percent antagonism was calculated as follows: $[1-(C-A/C-D)] \times 100$ where C is the number of stretches in mice treated with only PPQ, D is the number of stretches in mice with the drug and A is the number of stretches in mice with both antagonist and drug.

Measurement of total radioactivity in tissues

The radiolabelled drugs were diluted with unlabelled drug so that all animals would receive approximately 40 μCi kg^{-1} body weight. Rats and mice were injected s.c. with the appropriate dose of radiolabelled drug and then decapitated at 2 and 5 min, respectively.

Blood from the cervical wound was collected in heparinized tubes which were centrifuged at 1000 *g* for 20 min in order to obtain plasma. Brain and liver were homogenized in five volumes of 0.5 N hydrochloric acid using a polytron (Brinkmann Instruments, Inc., Westbury, NY). Total radioactivity was determined by counting 50 μl of plasma and tissue homogenates (50–100 μl) directly in aqueous counting scintillant (Amersham Corp., Arlington Heights, IL). Determination of total radioactivity in this fashion was verified by oxidizing some samples in a Packard Tri-Carb sample oxidizer and counting by liquid scintillation spectrometry. Quench was corrected by external standardization. Total radioactivity was expressed as pmol mg^{-1} of tissue by dividing the radioactivity in each sample by the specific activity of the injected material. Duncan's multirange test was used to test for significance among appropriate samples (Duncan, 1955).

Measurement of [^{3}H]-nicotine in tissue

An extraction technique that removed nicotine but not metabolites was used to quantitate [^{3}H]-nicotine (Tsujimoto, Nakashima, Tanino, Dohi & Kurogochi, 1975). Duplicate samples of homogenate (1 ml) and plasma (0.5 ml in rats and 0.3 ml in mice) were made basic with three drops of concentrated ammonium hydroxide and 1 ml of 40% K_3PO_4 (w/v). (−)-Nicotine di-L-tartrate (1 mg 200 μl^{-1}) was added as

a carrier and the samples were shaken with 10 ml of hexane for 0.5 h. The mixture was centrifuged at 3000 rev min^{-1} for 5 min, and 5 ml of the hexane was removed for counting in 10 ml of liquid scintillation composed of 227 ml of spectrofluor (2,5-diphenyloxazole - 1, 4 - *bis* [2-(4-methyl-5-phenyl-oxazolyl])benzene, Amersham) per gallon of toluene. Quench was corrected by using external standardization. We have demonstrated previously that greater than 90% of the [^{3}H]-nicotine is extracted by this procedure and that metabolites are not extracted (Tripathi, *et al.*, 1982). To ensure that methiodides or their metabolites were not removed by the hexane extraction, the remaining 5 ml of the hexane extracts were pooled for each tissue in each group, concentrated, and then analysed by thin-layer chromatography. The percentage of radioactivity corresponding to nicotine was then used to correct for the presence of methiodides and/or metabolites.

Results

Antinociception

As shown in Table 1, subcutaneous administration of nicotine produced appreciable antinociceptive activity in the tail-flick test in both species. These results are consistent with our previous studies in which we found that the ED_{50} (95% confidence limits) of nicotine was 12(7–21) μmol kg^{-1} in mice and 4.3 (2.5–6.7) μmol kg^{-1} in rats (Tripathi *et al.*, 1982). Doses of nicotine pyrrolidine methiodide that were twice the ED_{50} of nicotine showed little antinociceptive activity in both species. Higher doses could not be given due to the toxicity in mice. Severe tremors were also seen in rats when the doses exceeded 24 μmol kg^{-1}, s.c. The remaining quaternaries showed little antinociceptive activity even at very large doses. Antinociceptive activity was also measured after 5 and 10 min to rule out the possibility that the methiodides had a slower onset of action. All three methiodides were inactive at these later times.

As quaternization could render nicotine inactive, rather than merely preventing it from crossing the blood-brain barrier, mice were injected i.c.v. with nicotine and the methiodides in order to test for central antinociceptive activity. The data in Table 1 show that the pyrrolidine and *bis* methiodides, but not the pyridine methiodide, exert potent antinociceptive effects when injected i.c.v. Although the confidence limits for nicotine as well as the methiodides were very large (due to the shallowness of the dose-response curve) it appears that the pyrrolidine and *bis* methiodides may be more active than nicotine.

Since it is possible that peripheral administration of these quaternaries might produce antinociceptive activity using another assay, we decided to evaluate them in the commonly used mouse phenylquinone test. These results are summarized in Table 2. Again, nicotine was active and appeared to be more potent in this test system than in the tail-flick test. Nicotine pyridine methiodide was active at extremely high doses and nicotine *bis* methiodide never showed more than 50% inhibition of the stretching response. Nicotine pyrrolidine methiodide was considered more active than the other methiodides but was approximately half as potent as nicotine. Antagonism studies were then carried out to determine whether the pyrrolidine methiodide and nicotine produced antinociception through the same mechanism. The results in Table 2 show that hexamethonium blocked the antinociception of both agents to the same degree whereas mecamylamine antagonized nicotine com-

Table 1 *Tail-flick antinociceptive properties of nicotine and its methiodides in mice and rats*

	%effect or ED_{50}[a]	
	Mice	*Rats*
Treatment	*s.c administration (μ mol kg^{-1})*	
Nicotine	11 (6–21)	85% at 12
Nicotine pyrrolidine methiodide	5% at 10 and 4% at 20[b]	14% at 24[b]
Nicotine pyridine methiodide	0% at 440 and 1% at 900	17% at 300
Nicotine *bis* methiodide	4% at 440	14% at 300
	i.c.v. administration (nmol per mouse)	
Nicotine	152 (22–1,050)	—
Nicotine pyrrolidine methiodide	39 (21–72)	—
Nicotine pyridine methiodide	38% at 165	—
Nicotine *bis* methiodide	22 (1–360)	—

[a]Mice were tested 5 min after treatment and rats were tested 2 min after treatment.
[b]Severe tremors and death at higher doses.

Table 2 Antinociceptive action of nicotine and its quaternary methiodides in the mouse phenylquinone test after s.c. administration

Treatment	*ED_{50} (μmol kg^{-1})*	*%effect*	*%antagonism*
Nicotine	3.3 (2.0–5.5)		
Nicotine pyrrolidine methiodide	8.1 (4.9–13)		
Nicotine pyridine methiodide	900 (620–1,300)		
Nicotine *bis* methiodide	370[a]		
Nicotine alone[b]		87	
plus hexamethonium (3.7)[c]			53
plus hexamethonium (18)			66
plus mecamylamine (0.5)			28
plus mecamylamine (2.5)			97
Pyrrolidine methiodide[d]		74	
plus hexamethonium (3.7)			53
plus hexamethonium (18)			66
plus mecamylamine (0.5)			29
plus mecamylamine (2.5)			57
plus mecamylamine (10.0)			25
plus mecamylamine (20.0)			53

[a]Could not exceed 50% effect. [b]Dose of 5 μmol kg^{-1}. [c]μmol kg^{-1}. [d]Dose of 20 μmol kg^{-1}.

pletely but only partially blocked the antinociception produced by nicotine pyrrolidine methiodide.

Tissue levels of [^{3}H]-nicotine and [^{3}H]-methiodides

Drug disposition studies were carried out to determine the brain concentration of [^{3}H]-nicotine required for antinociceptive activity and to determine whether or not the methiodides penetrated brain following s.c. injection. The results of the distribution studies in mice are summarized in Table 3. Due to the fact that the doses of nicotine required to produce an ED_{50} in the tail-flick and phenylquinone tests were not comparable, the relationship between doses and brain concentrations was determined for nicotine. There was a dose-related increase in both plasma and brain levels of nicotine and it was readily apparent that nicotine easily penetrates brain. In addition, the nicotine metabolites (residual radioactivity) that were present in plasma did not enter brain to an appreciable extent.

Administration of [^{3}H]-nicotine pyrrolidine methiodide resulted in a very low level of total radioactivity in brain which was about 20 times less than that in plasma. [^{3}H]-nicotine pyrrolidine methiodide was metabolized to [^{3}H]-nicotine but only trace quantities of the latter were found in brain. A dose of [^{3}H]-nicotine pyridine methiodide equivalent to its ED_{50} in the mouse phenylquinone test resulted in appreciable quantities of radioactivity in brain. Approximately one-tenth of this radioactivity was due to [^{3}H]-nicotine. The radioactivity that was not extracted with hexane might contain a mixture of [^{3}H]-nicotine pyridine methiodide and metabolites. Metabolites may be present only in small quantities in light of the fact that nicotine metabolites do not penetrate brain to a great extent. Finally, [^{3}H]-nicotine *bis* methiodide in a dose that produces 50% inhibition in the phenylquinone test resulted in radioactivity in brain. Apparently, [^{3}H]-nicotine *bis* methiodide also is metabolized to [^{3}H]-nicotine which in turn enters brain in quantities sufficient to account for the antinociceptive activity of this methiodide. [^{3}H]-nicotine *bis* methiodide (residual radioactivity) also penetrated brain to a limited extent.

The distribution of [^{3}H]-nicotine and the methiodides was also studied in the brain and plasma of rats (Table 4). A dose of [^{3}H]-nicotine that produced 85% antinociceptive activity resulted in 2.1 pmol of [^{3}H]-nicotine mg^{-1} of brain 2 min after administration. Doses of [^{3}H]-nicotine pyrrolidine methiodide, [^{3}H]-nicotine pyridine methiodide and [^{3}H]-nicotine *bis* methiodide that were 2, 25, and 25 times greater than the dose of [^{3}H]-nicotine, respectively, resulted in brain levels of [^{3}H]-nicotine that were far less than that following [^{3}H]-nicotine administration. Apparently, the methiodides penetrate brain to a limited extent since there was no hexane-extractable radioactivity in brain following the administration of all three methiodides.

Table 3 Mouse brain and plasma levels of [^{3}H]-nicotine methiodides, [^{3}H]-nicotine and ^{3}H-residual radioactivity after s.c. administration[a]

Treatment	*Dose* (μmol kg^{-1})	*Total radioactivity*		*[^{3}H]-nicotine*		*^{3}H-Residual*	
		Brain	*Plasma*	*Brain*	*Plasma*	*Brain*	*Plasma*
[^{3}H]-nicotine	1.5	1.4±0.6	1.1±0.1	1.6±0.7	0.4±0.05	—	0.6±0
	3.1	3.2±0.1	2.7±0.1	3.2±0.1[A]	1.1±0.2[C]	0.0±0	1.4±0.1
	6.2	5.7±0.2	5.8±0.6	5.6±0.9	2.0±0.5	0.1±0.1	3.1±0.2
	10.7	11.0±1.0	8.3±1.0	10.7±0.9[B]	3.5±0.6[D]	0.2±0.2	4.9±0.5
[^{3}H]-nicotine pyrrolidine methiodide	8.0	0.1±0.0	1.9±0.1	0.02±0.00[b,c]	0.01±0.0[d,e]	0.1±0.0	1.9±0.1
[^{3}H]-nicotine pyridine methiodide	900.0	20.5±3.5	787±03.7	1.8±0.2[b,c]	0.6±0.2[e]	19.5±3.4	786±931
[^{3}H]-nicotine *bis* methiodide	370.0	6.0±0.9	339±16.9	2.7±0.2[c]	2.4±0.4[d,e]	4.3±0.8	337±17

[a]Data expressed as pmol mg^{-1} tissue and means ± s.e. mean (n = 6) are presented.
[b]Significantly different from A at $P < 0.05$ by Duncan multirange test.
[c]Significantly different from B at $P < 0.05$ by Duncan multirange test.
[d]Significantly different from C at $P < 0.05$ by Duncan multirange test.
[e]Significantly different from D at $P < 0.05$ by Duncan multirange test.

Table 4 Rat brain and plasma levels of [^{3}H]-nicotine, [^{3}H]-nicotine methiodides and ^{3}H-residual radioactivity after s.c. administration[a]

Treatment	*Dose* (μmol kg^{-1})	*Total radioactivity*		*[^{3}H]-nicotine*		*^{3}H-residual*	
		Brain	*Plasma*	*Brain*	*Plasma*	*Brain*	*Plasma*
[^{3}H]-nicotine	12	2.20±.20	3.3±.20	2.1±.20	1.9±.20	0.12±.10	1.54±.12
[^{3}H]-nicotine pyrrolidine methiodide	24	0.23±.03	8.13±1.32	0.03±.00[b]	0.03±.00[b]	0.23±.03	8.10±.13
[^{3}H]-nicotine pyridine methiodide	300	3.49±.26	107±6.56	0.36±.03[b]	0.19±.03[b]	3.13±.26	107±6.56
[^{3}H]-nicotine *bis* methiodide	300	3.10±.29	117±6.8	0.29±.02[b]	0.11±.01[b]	2.89±.27	117±6.8

[a]Data expressed as pmol mg^{-1} tissue, means ± s.e. mean of n = 5.
[b]Significantly different from nicotine-treated rats at $P < 0.01$ by Duncan multirange test.

Discussion

All three methiodides were inactive in the mouse tail-flick procedure when administered peripherally at doses which exceeded the ED_{50} dose of nicotine by 2, 40, and 40 times in the case of the pyrrolidine, pyridine and *bis* methiodides, respectively. The high toxicity of nicotine pyrrolidine methiodide was consistent with the finding of Larson & Haig (1943). The lack of antinociceptive activity of the methiodides in this test was also consistent with our hypothesis that nicotine was producing this effect through a central mechanism. The nicotine pyrrolidine and *bis* methiodides were active when injected directly into the ventricles which demonstrated that had they penetrated brain after subcutaneous administration, they would have shown antinociceptive activity. The results from the disposition experiments show that some radioactivity does indeed appear in brain following the administration of the radiolabelled methiodides. It is reasonable to assume that the radioactivity in brain is predominantly the parent compound since metabolites of the methiodides would probably be even more polar and less likely to penetrate brain. The brain levels of nicotine *bis* methiodide and nicotine pyrrolidine methiodide are much less than the nicotine levels which produce 50% antinociception. The brain levels of nicotine pyridine methiodides and/or its metabolites were twice as high as those of nicotine, yet there was no antinociceptive activity. This latter finding is consistent with the lack of antinociceptive activity of the pyridine methiodide following i.c.v. administration. A potential complication with the study of these methiodides is their possible metabolism to nicotine which in turn could easily penetrate brain. McKennis, Bowman, Hovath & Bederka (1964) have shown that a series of pyridinium methiodides can be N-demethylated. Also, Thompson *et al.*, (1972) have tried with no success to measure nicotine levels in rat brain after administration of nicotine pyrrolidine methiodide. We found nicotine in mouse brain after administration of the methiodides in levels that constituted approximately one-tenth and one-half of the brain radioactivity of the pyridine and *bis* methiodides, respectively. However, these levels of nicotine were insufficient to produce antinociceptive activity. It would appear that the antinociceptive activity of nicotine, as measured by tail-flick, is of central origin since the methiodides were inactive when administered peripherally. These conclusions are consistent with our previous finding that nicotine brain levels and antinociception correlates well in mice (Tripathi *et al.*, 1982).

Since it has been shown that the mechanism of morphine antinociception differs in the phenylquinone and tail-flick tests (Aceto, Martin & Tucker, 1981), it would be reasonable to assume that a similar situation could exist for other drugs, including nicotine. Studies were carried out to determine if nicotine's antinociceptive actions in the phenylquinone test were also centrally mediated. All three methiodides were active in the phenylquinone test, although they were 3 to 300 times less potent than nicotine. The weak antinociceptive activity of the *bis* methiodide in phenylquinone test can be explained on the basis of its metabolism to nicotine. ED_{50} doses of nicotine *bis* methiodide and nicotine ($3.1\ \mu mol\ kg^{-1}$) result in comparable brain levels of nicotine. The ED_{50} dose of the pyridine methiodide resulted in brain levels of nicotine that were half those following the ED_{50} dose of nicotine. It would appear that the pyridine methiodide has weak central and/or peripheral antinociceptive properties in the phenylquinone test but not in the tail-flick test. The trace quantities of radioactivity in brain following administration of [^{3}H]-nicotine pyrrolidine methiodide clearly show that its antinociceptive action in the phenylquinone test must be peripherally mediated. These apparently conflicting results with the methiodides in this antinociceptive test could be interpreted to mean that this action of nicotine is peripherally mediated and the methiodides merely differ in potency. Peripherally active analgesics have been found to be active in the phenylquinone test (Hendershot & Fursaith, 1959) but usually not in the tail-flick procedure. However, the fact that mecamylamine only partially blocked the antinociceptive action of nicotine pyrrolidine methiodide suggests that this compound has both nicotine and non-nicotine antinociceptive actions. These data do not rule out the possibility that the antinociceptive activity of nicotine in the mouse phenylquinone test is centrally mediated, at least in part. This conclusion is supported by the finding that hexamethonium only partially blocks nicotine antinociception (Table 2).

The methiodides were much less active than nicotine in the rat tail-flick test. Nicotine pyrrolidine methiodide was inactive at twice the ED_{50} dose of nicotine and the other two methiodides were inactive at doses that were 25 times higher. These doses of radiolabelled methiodides resulted in only trace quantities of radioactivity in brain after nicotine pyrrolidine methiodide, whereas the brain levels of the other two methiodides were higher than the brain levels of nicotine following nicotine administration. Therefore, it would appear that the pyridine and *bis* methiodides are less active than nicotine. Only trace quantities of nicotine were found in rat brain following administration of the methiodides which indicates a metabolic difference between mice and rats.

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