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Some central effects in mice of compounds related to nicotine

R. B. BARLOW*, A. OLIVERIO†, M. SATTA† AND G. M. THOMPSON*

** Department of Pharmacology, University of Edinburgh ; † Laboratorio di Psicobiologia e Psicofarmacologia del C.N.R., Via Reno 1, Rome, and Department of Pharmacology, University of Sassari*

Summary

1. Some hydroxy-, amino-, and methoxy- phenylalkyltrimethylammonium compounds, β-pyridylmethyl- dimethylamine and pyrrolidine, and β-pyridylethyltrimethylammonium, were tested on avoidance learning in mice and their effects were compared with those of (−)-nicotine.

2. The *o*- and *m*- hydroxybenzyl-, *o*-hydroxyphenethyl- and *m*-hydroxyphenylpropyl- trimethylammonium compounds improved performance ; (−)-nicotine, in one-quarter of the dose, had similar effects. The *m*- and *p*-hydroxyphenethyl-, *o*-hydroxyphenylpropyl- and *o*- and *p*- aminobenzyl, and *o*-, *m*-, and *p*- aminophenethyl-trimethylammonium compounds impaired performance.

3. (−)-Nicotine and *m*-hydroxyphenylpropyltrimethylammonium appeared also to enhance memory consolidating processes.

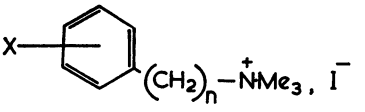
4. The central actions of some of the compounds suggest that the possibility that they can penetrate into the central nervous system should not be ruled out even though they are quaternary salts.

5. No correlation was found between the effects of the compounds on avoidance learning and on the frog rectus muscle. Though the differences may be due to differences in access to the central nervous system, it is also possible that the receptors associated with learning processes are different from those in the frog rectus and possibly more specialized.

Introduction

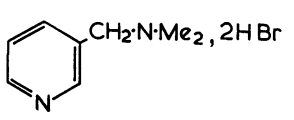
(−)-Nicotine has been shown to facilitate avoidance learning (Bovet & Bovet-Nitti, 1965 ; Bovet, Bovet-Nitti and Oliverio, 1966 ; Oliverio, 1966). This paper describes the effects on avoidance learning of some analogues of nicotine, prepared and tested by Barlow & Thompson (1969) on the nicotine-sensitive receptors of the rectus abdominis muscle of the frog, *Rana pipiens*, on which some of them were highly active.

The compounds had the structure:



Where X was *o*-, *m*- and *p*- OH and n=1, 2 and 3 ; *o*-, *m*- and *p*- NH_2 and n=1 and 2 ; *o*-, *m*- and *p*- OMe and n=1 and *o*- and *m*- OMe and n=2 and 3.

We also tested three compounds which contain the β-pyridyl ring present in nicotine, two of these were tertiary bases:



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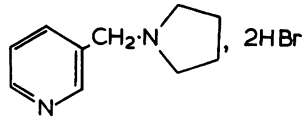
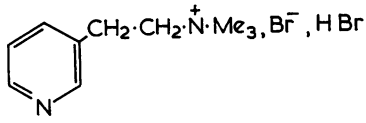


The third was the quaternary salt:



Methods

The mice used were males, strain DBA/2J (the Jackson laboratory); which weighed between 22 and 25 g. The drugs were dissolved in 0·9% sodium chloride and were injected intraperitoneally 15 min before testing. The mice were divided randomly into groups of eight and each group received the same dose of the same drug each day for the 5 consecutive days on which tests were made.

Nicotine was tested in doses of 0·25, 0·50 and 1·00 mg (base)/kg and the other compounds in doses of 1·0 and 2·0 mg/kg. Control mice were injected with 0·9% sodium chloride and in all the mice the volume of solution injected, saline or drug, was 0·4 ml/100 g body weight. (−)-Nicotine hydrogen tartrate was obtained from Baker Chemical Co. (N.J.). The analytical purity of the other compounds has been described by Barlow & Thompson (1969).

Acquisition of a conditioned avoidance response

The experiments took place in two sound-proof compartments (Mercury Cabin 303) each containing eight shuttle-boxes. The apparatus consisted of a rectangular white Plexiglass box (40 by 10 cm) divided into two equal compartments connected by a small opening (3 by 3 cm) through a black partition (Bovet *et al.*, 1966). The floor consisted of stainless steel rods spaced 0·4 cm apart. On each trial the conditioned stimulus, a light (10 W), preceded by 5 s the unconditioned stimulus, a continuous shock (1·5 mA) through the grid floor. The mice avoided the shock by running into the adjacent compartment within 5 s of the onset of the conditioning stimulus. The interval between trials was 30 s. Spontaneous crossings were punished and recorded as "inter-trial" responses. These did not occur often—usually only one or two such responses occurred with a few mice during the first session only. Before each session each mouse was left alone in the apparatus for 10 min to become adapted to its surroundings. The stimuli were delivered by means of an automated programming device to alternate sides of the shuttle-box and the responses were recorded automatically. The technique is described in detail by Bovet, Bovet-Nitti & Oliverio (1969).

Each group of mice was given 100 trials/day for each of the 5 consecutive days of the test. During this period the animals learned to respond to the conditioning stimulus and the number of times when they avoided the subsequent shock rose, as

can be seen in Table 1. To observe the effects of drugs, the number of avoidances during the five sessions with the test group were compared with the number observed with the controls and tested for significance by chi-square tests (Siegel, 1956).

Some experiments were also made with " post-trial " injections in which nicotine, 0·5 mg(base)/kg and *m*-hydroxypropyltrimethylammonium iodide, 2·0 mg/kg, were injected into groups of eight mice immediately after each training session. The animals were then tested 24 h later, without any further drug treatment, but were again injected after the test, and so on for 5 consecutive days. Control animals were also tested which had only received saline as a post-trial injection. Because the drugs are likely to have been eliminated during the 24 h period, any effects on performance are likely to be due to the effects of the compounds on memory consolidation processes (McGaugh & Petrinovich, 1965). As in the other experiments, the numbers of avoidances with the treated animals were compared with those with the controls and tested for significance by chi-square tests.

Results

Effects of (−)-nicotine

The results are shown in Table 1. There is a significant improvement in avoidance behaviour with the lower doses (0·25 and 0·50 mg/kg, $P<0·01$), but the higher dose (1·0 mg/kg) significantly impaired performance ($P<0·01$).

Effects of the analogues of nicotine

The results are shown in Table 2 and summarized in Table 3. The methoxy compounds and the β-pyridyl compounds had no significant effects. The amino compounds had no significant effect in the low dose and impaired performance in the high dose, except for *m*-aminobenzyltrimethylammonium, which had no significant effect at any dose. With the hydroxy- compounds various effects were observed. The *o*- and *m*- hydroxybenzyl compounds ($n=1$) and the *m*-hydroxyphenylpropyl compound ($n=3$) improved avoidance behaviour in both the low and high doses, but the analogous *p*-hydroxy-benzyl and phenylpropyl compounds ($n=1$ and 3) were without significant effect. The *m*- and *p*- hydroxyphenethyl compounds ($n=2$) impaired avoidance behaviour in both doses. The *o*-hydroxyphenethyl compound ($n=2$) markedly improved the level of avoidances in the high dose, but was ineffective in the low dose. The *o*-hydroxyphenylpropyl compound ($n=3$), on the other hand, resembled the amino-compounds in that it was ineffective in low doses and impaired avoidance behaviour in high doses.

Effects of post-trial injections

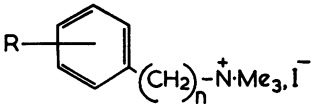
With the groups of mice which received nicotine or *m*-hydroxyphenylpropyltrimethylammonium iodide immediately after each session of 100 trials there was a higher number of avoidances than with the saline-treated controls. The mean results for the five consecutive sessions were: controls $40·77 \pm 1·91$, nicotine 0·5 mg(base)/kg $47·35 \pm 2·10$; *m*-hydroxyphenylpropyltrimethylammonium iodide 2·0 mg/kg, $48·21 \pm 2·33$. The differences from the controls were significant ($P<0·01$).

TABLE 1. *Effects of (−)-nicotine on avoidance learning*

	Dose (mg/kg)	Sessions 1	2	3	4	5	1–5
Controls		8·15±0·51	29·42±1·98	39·41±2·14	56·17±2·04	74·12±1·80	41·45±2·13
Nicotine	0·25	9·10±1·04	34·18±1·36	45·13±1·89	58·74±3·71	78·12±2·33	45·05±1·78*
	0·50	8·71±0·90	38·71±1·24	48·56±2·31	68·88±2·87	82·10±3·07	49·39±2·31*
	1·00	7·18±1·01	18·10±0·97	25·38±1·01	36·75±2·11	50·12±2·04	27·50±1·61*

The numbers represent the mean % avoidances±S.E. Results marked with an asterisk are significantly different from the controls ($P<0·01$).

TABLE 2. *Mean % avoidance*±S.E. *during sessions* 1–5



Controls 41·45±2·13

	o		*m*		*p*	
	1 mg/kg	2 mg/kg	1 mg/kg	2 mg/kg	1 mg/kg	2 mg/kg
R = OH						
n = 1	47·65±2·41**	49·00±2·11*	48·55±1·96*	49·17±2·01*	37·55±3·15	38·30±4·12
n = 2	38·50±2·08	56·30±2·81*	33·59±2·01*	32·59±1·75*	33·59±2·98*	35·80±3·07**
n = 3	42·98±1·65	32·40±2·54*	48·00±2·83**	52·54±2·54*	40·25±2·01	44·40±3·12
R = NH_2						
n = 1	37·25±3·27	21·00±1·06*	46·70±1·96	44·99±1·96	43·95±3·12	32·58±1·15*
n = 2	40·21±1·98	22·14±1·12*	43·50±3·01	27·64±1·07*	41·33±1·89	27·84±2·01*
R = OMe						
n = 1	40·10±2·05	41·64±2·37	39·55±1·65	40·88±2·15	36·92±3·10	41·23±1·64
n = 2	40·98±3·61	37·68±3·12	39·51±2·06	42·03±3·61		
n = 3	41·66±2·64	37·19±3·01	41·15±1·98	40·67±1·98		
β-pyridylmethyldimethylamine dihydrobromide			41·16±2·10	39·11±2·00		
β-pyridylmethylpyrrolidine dihydrobromide			42·11±1·00	40·45±2·16		
β-pyridylethyltrimethylammonium bromide hydrobromide			40·36±1·38	39·76±2·11		

One asterisk indicates significance at the level $P<0·01$; two asterisks indicate significance at the level $P<0·05$.
Each group represents the mean of eight mice. The performance of the control group is reported in Table 1.

Reproducibility of controls

The mean percentage of avoidances for the controls in the experiments with post-trial injections ($40{\cdot}77 \pm 1{\cdot}91$) is not significantly different from the value for the original controls ($41{\cdot}45 \pm 2{\cdot}13$), and in a subsequent control group the value was $40{\cdot}83 \pm 2{\cdot}98$.

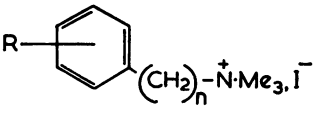
Discussion

It is remarkable that some of the quaternary ammonium salts we have tested produce effects on avoidance behaviour 15 min after intraperitoneal injection. Although impairment of performance could well be due to a peripheral action of the drugs, it is more difficult to explain the improvement of the level of avoidances by a peripheral action. It also seems clear from the results of the " post-trial " experiments that *m*-hydroxyphenylpropyltrimethylammonium produces effects on memory consolidating processes. Although it is not absolutely impossible that such central effects may be peripheral in origin, it seems advisable to consider whether the compound may penetrate into the central nervous system, even though it is a quaternary salt. Direct information about this might be obtained by studying the distribution of an isotopically labelled form of the compound.

In view of the activity of some of the quaternary salts, it was surprising that the tertiary bases were inactive. The compound β-pyridylmethylpyrrolidine is isomeric with nicotine and has a similar pKa (Barlow & Hamilton, 1962). It seems highly likely that this molecule can penetrate to any site accessible to nicotine. Neither of the tertiary bases tested, however, was very active at peripheral nicotine-sensitive receptors and their lack of effect on avoidance behaviour seems likely to be due to their feeble activity at the receptors associated with this action of nicotine.

A comparison of the results on avoidance behaviour with those obtained on the nicotine-sensitive receptors of the frog rectus muscle is shown in Table 3. There seems to be no relationship between the two. Compounds with the same peripheral

TABLE 3. *Comparison of results on avoidance learning and on the frog rectus preparation*



	o			*m*			*p*		
	1 mg/kg		2 mg/kg	1 mg/kg		2 mg/kg	1 mg/kg		2 mg/kg
R = OH									
n = 1	+	(2·4)	+	+	(1·7)	+	o	(2·4)	o
n = 2	o	(2·4)	+	−	(−0·25)	−	−	(0·7)	−
n = 3	o	(1·6)	−	+	(−0·7)	+	o	(1·3)	o
R = NH_2									
n = 1	o antagonist		−	o	(2·7)	o	o partial agonist		−
n = 2	o	(0·6)	−	o	(0·3)	−	o	(−0·4)	−
β-pyridylethyltrimethylammonium bromide hydrobromide				o	(−0·23)	o			

o indicates no effect, + indicates improved performance, − indicates impaired performance. The numbers in parenthesis indicate the log. of the equipotent molar ratio relative to β-pyridylmethyltrimethylammonium on the frog rectus. Positive values indicate that more material is needed and the compound is therefore weaker than the standard, negative values indicate that less material is needed to produce the same effect and it is therefore more active than the standard.

activity, such as *o*- and *p*- hydroxybenzyltrimethylammonium and *o*- hydroxyphenethyltrimethylammonium, do not have the same effects on avoidance behaviour. The most active compound on the frog rectus, *m*-hydroxyphenylpropyltrimethylammonium, enhanced learning but the next most active compounds, *p*-aminophenethyltrimethylammonium and *m*-hydroxyphenethyltrimethylammonium (Leptodactyline, Erspamer & Glässer, 1960), impaired performance. In contrast β-pyridylethyltrimethylammonium was without effects.

The differences in the results with the two tests may occur because of the difficulty with which some compounds penetrate into the central nervous system, but it is also possible that they arise because the receptors affected are different. It has been suggested that receptors in mammals should be more specialized than those in amphibia and receptors in the central nervous system more specialized than those in peripheral tissues (Ginetsinskii, 1947; Koelle, 1962; Mikhel'son & Fruentov, 1963; Magazani, Fruentov, Roshkova, Rybolovlev & Mikhel'son, 1965). This would explain why fewer compounds were active in the test on avoidance learning in mice than were active at the peripheral level.

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