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Investigation of central cholinergic mechanisms in the conscious mouse

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Summary

1. An investigation of central cholinoceptors in the mouse has been made by injecting cholinomimetic drugs into the cerebral ventricles and seeing how their effects were modified by prior administration of atropine-like substances and other drugs.

2. Carbachol or oxotremorine injected in small doses intracerebroventricularly into conscious mice caused hypothermia, gross tremor and a variety of parasympathomimetic effects including lachrymation and salivation. Acetylcholine injected in this way was active only in much larger doses.

3. Methacholine and pilocarpine also caused a variety of parasympathomimetic effects after intracerebroventricular injection but virtually no hypothermia or tremor.

4. Nicotine injected intracerebroventricularly caused mild hypothermia, fine tremor but no parasympathomimetic effects.

5. Atropine-like drugs, tricyclic antidepressants and amphetamine antagonized the hypothermia induced by intracerebroventricular carbachol or oxotremorine.

6. The sites of action of the atropine-like drugs are in the brain ; those of the tricyclic antidepressants and amphetamine are in the periphery probably on heat generating β-adrenoceptor mechanisms.

7. It is concluded that the atropine sensitive cholinoceptors in the brain vary in their sensitivities to cholinomimetic drugs, other than acetylcholine, and may exist in isoreceptor forms.

8. Peripheral atropine sensitive cholinoceptors may also exist in isoreceptor forms.

Introduction

From studies in which catecholamines were injected into the cerebral ventricles of conscious mice, Brittain & Handley (1967) concluded that the brain might contain adrenoceptors analogous to the α- and β-adrenoceptors at the periphery. A similar investigation of central cholinoceptors has now been made by injection of cholinomimetic agents into the cerebral ventricles of conscious mice and seeing how the observed effects were modified by previous administration of atropine-like substances and other drugs. Oxotremorine, methacholine and pilocarpine were chosen as cholinomimetic agents because of their predominently muscarinic activity at the periphery, and carbachol because of its mixed muscarinic and nicotinic effects.

Some of this work was presented to the British Pharmacological Society (Ankier, Brittain & Handley, 1968).

Methods

Groups of not less than five male albino mice (Glaxo A_2G strain), each weighing 20–25 g, were kept for at least 18 h before the experiment in a temperature controlled room at 19–21° C.

Temperatures were measured using a calibrated electric thermometer (Type 3GID–2752; range 15/45° C; Light Labs., Brighton). Body temperature was determined using a thermocouple mounted in an 18 gauge needle which was briefly inserted into the oesophagus until a constant temperature reading was obtained (Brittain & Spencer, 1964). Skin temperature was determined by placing a temperature probe (Type TO 2420; range 19/45° C; Light Labs., Brighton) lightly but firmly on the ventral surface of a hind paw for not less than 5 seconds. In studies on the interaction of drug effects, oesophageal temperatures were measured 30 min before and 30 min after the intracerebroventricular injections. The percentage inhibition of hypothermia induced by a cholinomimetic agent was calculated using the formula $\frac{(x_2-x_1)-(y_2-y_1)}{(x_2-x_1)} \times 100$ where x_2 and x_1 are the differences in oesophageal temperature caused by the intracerebroventricular injection of a cholinomimetic agent and saline respectively, and y_2 and y_1 are the corresponding values in the groups pretreated with atropine-like substances or other drugs. The dose of drug which inhibited the hypothermic effect by 50% (ID50 value) was calculated by the method of Fieller (1940).

Drugs

Drugs for intracerebroventricular injection were dissolved in 0·9% (w/v) apyrogenic sodium chloride solution and injected in a dose-volume of 0·02 ml/mouse following the method of Ankier (1969). For oral administration, drugs were dissolved or suspended in 5% (w/v) gum acacia and given in a volume of 0·4 ml/20 g body weight 1 h before intracerebroventricular injection of the cholinomimetic agents. For subcutaneous injections drugs were injected in a volume of 0·2 ml/20 g 1 h before an intracerebroventricular injection.

The drugs included: acetylcholine chloride, amitriptyline hydrochloride, (±)-amphetamine sulphate, atropine methonitrate, atropine sulphate, carbachol chloride, chlordiazepoxide hydrochloride, chlorpromazine, methaqualone hydrochloride, morphine hydrochloride, nicotine tartrate, nortriptyline hydrochloride, oxotremorine, pentobarbitone sodium, perphenazine hydrochloride, phenelzine dihydrogen sulphate, phenytoin sodium and propantheline bromide. The doses in the text refer to free acid or base.

Results

Effects of intracerebroventricular injections of acetylcholine, carbachol, oxotremorine, pilocarpine, methacholine and nicotine

Acetylcholine

Acetylcholine, in doses as high as 20 μg/mouse, had no obvious effects on oesophageal temperature or behaviour. Higher doses (50–200 μg/mouse) caused

some gross tremor and lachrymation and a relatively small dose dependent fall in body temperature. For example, at 80 μg/mouse, acetylcholine lowered body temperature by $2{\cdot}5 \pm 0{\cdot}8°$ C after 30 min ; recovery took about 2 hours.

Carbachol

Carbachol (0·1–2·0 μg/mouse) caused brief intense salivation and lachrymation, bradypneoa and pronounced dose dependent falls in body temperature which were preceded by short-lasting peripheral vasodilatation as shown by a rise in skin temperature (Fig. 1). Some gross tremor was also produced and with larger doses convulsions occurred ; the intracerebroventricular LD50 was 7·5 (5·7–9·5) μg/mouse.

These and even larger doses of carbachol given intravenously caused only minimal salivation and lachrymation and no hypothermia or tremor.

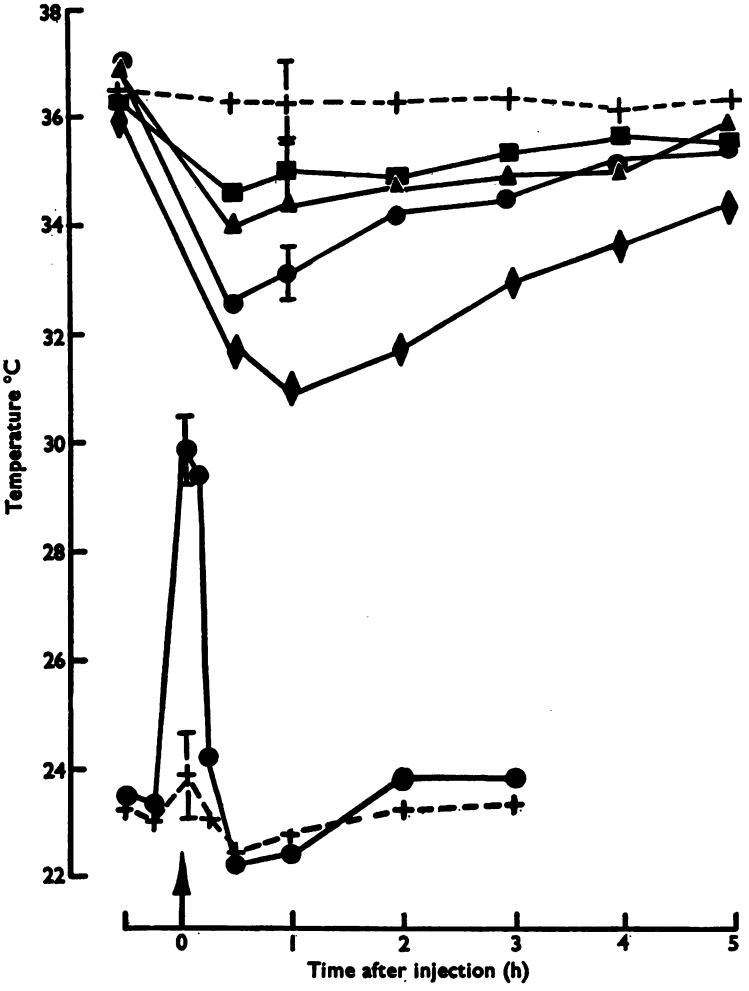

FIG. 1. Effects of intracerebroventricular injection of carbachol on oesophageal (upper set of curves) and skin temperatures (lower set of curves) in the conscious mouse. Doses (μg/mouse): 0·5, —■— ; 1, —▲— ; 2, —●— ; 5, —◆—. Saline solution —+—.

Oxotremorine

Oxotremorine (0·1–2·0 μg/mouse) caused intense short lasting lachrymation and tremor, prolonged salivation and hypothermia. The tremor was more vigorous but shorter lasting than after carbachol, was maximal approximately 15 min after injection and its onset slightly preceded the hypothermia. The hypothermia was of shorter duration than that of carbachol probably because oxotremorine is less stable in the body. Even with a temperature fall as great as 5° C, recovery was complete in about 2 h (Fig. 2). As with carbachol the main heat loss occurred shortly after the intracerebroventricular injection as shown by a pronounced rise in skin temperature (Fig. 2). Relatively high intracerebroventricular doses of oxotremorine were well tolerated, the intracerebroventricular LD50 was 67·2 (59·7–74·2) μg/mouse.

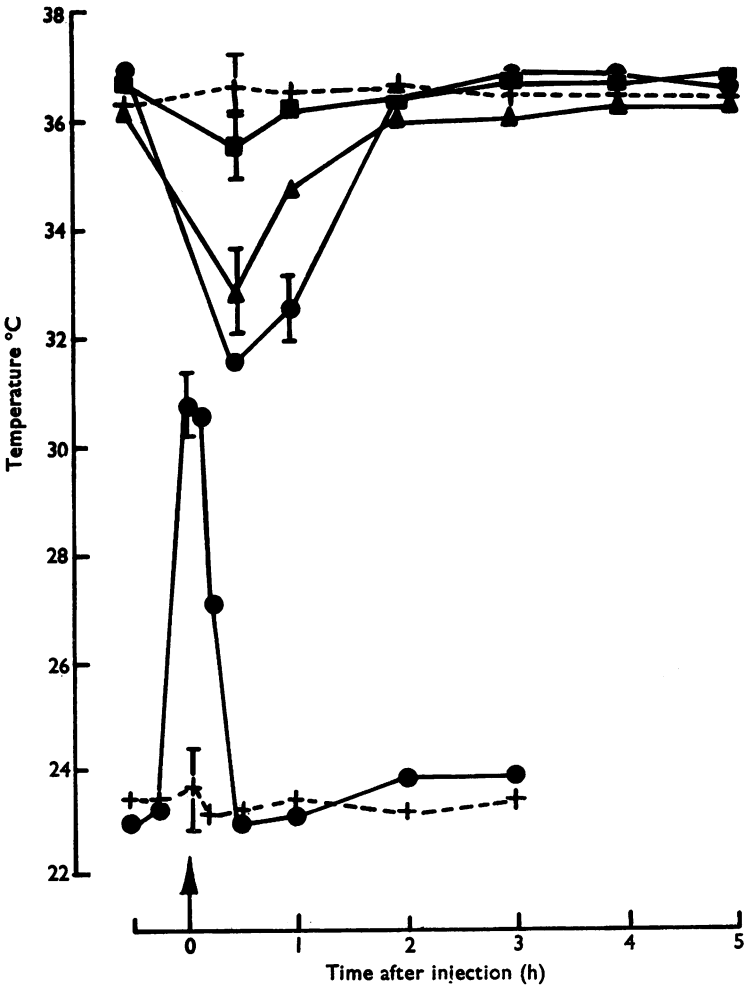

FIG. 2. Effects of intracerebroventricular injection of oxotremorine on oesophageal (upper set of curves) and skin temperatures (lower set of curves) in the conscious mouse. Doses (μg/mouse): 0·5, —■—; 1, —▲—; 2, —●—. Saline solution —+—.

Pilocarpine and methacholine

Both drugs (50–200 μg/mouse) caused pronounced lachrymation and salivation, slight transient hypothermia and no tremor. The intracerebroventricular LD50's for pilocarpine and methacholine were greater than 200 μg/mouse.

Nicotine

Nicotine (0·1–2·0 μg/mouse) caused slight transient hypothermia, some fine tremor but neither lachrymation nor salivation. The tremor was of a quivering nature and was distinctively different from the more gross tremor induced by carbachol and oxotremorine. Higher doses of nicotine produced convulsions followed by depression ; the intracerebroventricular LD50 was 10·2 (7·1–14·0) μg/mouse.

The comparative effects of the cholinomimetic agents following intracerebroventricular injection are summarized in Table 1.

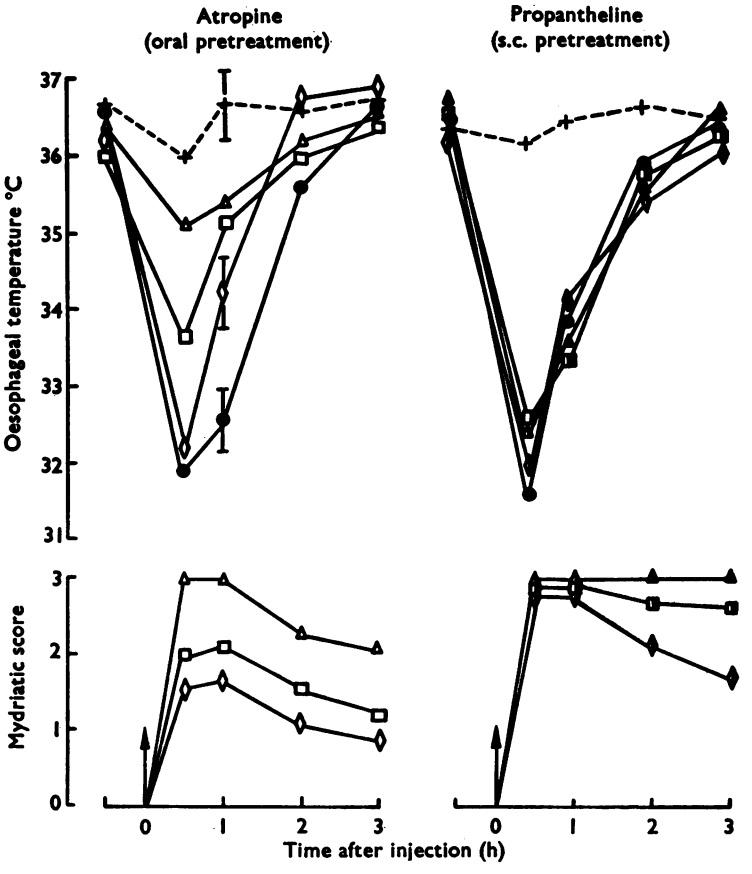

FIG. 3. Effects of atropine sulphate and propantheline in inhibiting hypothermia induced by intracerebroventricular injection of oxotremorine and in causing mydriasis in the conscious mouse. Mydriasis was assessed visually: 0, no effect ; 1, slight effect ; 2, moderate effect ; 3, intense effect. Atropine sulphate doses (mg/kg) orally: 1, —◇—; 2, —□—; 4, —△—. Propantheline doses (mg/kg) subcutaneously: 4, —◁▶—; 8, —◨—; 16, —◭—. Oxotremorine control, 2 μg/mouse, —●—. Saline solution —+—.

Effects of drugs on the responses to intracerebroventricular injection of carbachol and oxotremorine

Atropine and atropine-like substances

Atropine sulphate, whether given orally (5 mg/kg) or injected intracerebroventricularly (1 μg/mouse) abolished all the responses obtained with intracerebroventricular injections of the cholinomimetic drugs. In contrast, the quaternary atropine-like substances, atropine methonitrate and propantheline, which do not readily enter the brain on systemic administration, affected the responses to carbachol and oxotremorine differently according to the route of application. Injected subcutaneously (4 mg/kg) only the responses mediated through parasympathetic fibres, such as lachrymation and salivation, were abolished, whereas tremor and hypothermia were not affected. Injected intracerebroventricularly (2–4 μg/mouse)

TABLE 1. *Comparison of the effects after intracerebroventricular injection of acetylcholine, carbachol, oxotremorine, pilocarpine, methacholine and nicotine*

Drug	Dose range (μg/mouse)	Intensity of effects observed: Hypothermia	Tremor	Salivation	Lachrymation	LD50* values with 95% fiducial limits (μg/mouse)
Acetylcholine	50–200	+	+	0	+	>200
Carbachol	0·1–2·0	+++	+	+++	+++	7·5 (5·7–9·5)
Oxotremorine	0·1–2·0	+++	+++	++	+++	67·2 (59·7–74·2)
Pilocarpine	50–200	+	0	+++	+++	>200
Methacholine	50–200	+	0	++	+++	>200
Nicotine	0·1–2·0	+	+	0	0	10·2 (7·1–14·0)

0=No effect; + slight effect; ++=moderate effect; +++=pronounced effect. *LD50 values (1 h) with their 95% fiducial limits calculated by logit analysis.

TABLE 2. *Inhibitory effects of drugs on the hypothermia induced by intracerebroventricular injection of carbachol or oxotremorine in conscious mice*

Drug	ID50 value (mg/kg) to inhibit hypothermia induced by intracerebroventricular injection: Carbachol	Oxotremorine
Centrally and peripherally acting atropine-like substances		
Atropine sulphate	2·0 (1·3–3·0)	1·9 (0·9–3·4)
Atropine methonitrate	Inactive (20)	
Propantheline	Inactive (20)	
Anti-depressant monoamine oxidase inhibitors and stimulants		
Imipramine	9·5 (6·5–14·0)	9·1 (5·3–13·3)
Amitriptyline	8·5 (4·5–12·5)	8·0 (3·7–12·0)
Nortriptyline	14·0 (5·6–22·8)	19·0 (8·8–30·8)
Phenelzine	Inactive (20)	
Amphetamine	2·9 (1·2–4·3)	2·4 (1·3–4·8)
Neuroleptics		
Chlorpromazine	Inactive (1–50)	
Perphenazine	Inactive (1–50)	
Haloperidol	Inactive (1–50)	
Hypnotics and anticonvulsants		
Phenobarbitone	Inactive (5–20)	
Methaqualone	Inactive (5–20)	
Phenytoin	Inactive (20)	
Tranquillizer		
Chlordiazepoxide	Inactive (5–20)	
Analgesics		
Codeine	Inactive (20–50)	
Morphine	Inactive (20–50)	

Drugs were given orally 1 h before intracerebroventricular injection of carbachol (2 μg/mouse) or oxotremorine (2 μg/mouse) except atropine methonitrate and propantheline which were administered subcutaneously. Figures in parentheses indicate the doses or dose ranges investigated.

all the responses including tremor and hypothermia were abolished. The effects of systemically administered atropine sulphate and propantheline on hypothermia caused by intracerebroventricular injection of oxotremorine are shown in Fig. 3. This figure also indicates the degree of mydriasis produced by the different doses of atropine and propantheline. Despite the pronounced and more sustained mydriasis produced by propantheline it did not affect the hypothermia produced by oxotremorine.

In Tables 2 and 3, the inhibitory effects of atropine sulphate, atropine methonitrate and propantheline on the hypothermia produced by intracerebroventricular carbachol or oxotremorine are expressed as ID50. The results obtained on oral or subcutaneous administration of these antagonists are given in Table 2 and on intracerebroventricular administration in Table 3. The high activity of these drugs after intracerebroventricular injection and the inactivity of the quaternary compounds after systemic administration suggests that their sites of action are in the brain.

Other drugs

The effects of various drugs other than atropine-like substances on hypothermia caused by carbachol and oxotremorine are also summarized in Tables 2 and 3. The tricyclic antidepressant drugs and amphetamine were only active after systemic administration and at doses which did not cause mydriasis. This suggests that the inhibition is a peripheral effect and does not involve cholinoceptors.

Discussion

The finding that acetylcholine injected intracerebroventricularly was poorly active in comparison with the more stable cholinomimetic drugs oxotremorine and carbachol is readily explained by the high acetylcholinesterase content of the brain (Augustinsson, 1948 ; Ord & Thompson, 1950 ; Gerebtzoff, 1959 ; Moerman, 1963). The effects obtained with the cholinomimetic drugs using this intracerebroventricular

TABLE 3. *Inhibitory effects of drugs administered intracerebroventricularly on the hypothermia induced by the intracerebroventricular injection of carbachol or oxotremorine in conscious mice*

Drug	ID50 value (μg/mouse) to inhibit hypothermia induced by intracerebroventricular injection	
	Carbachol	Oxotremorine
Atropine sulphate	0·43 (0·28–0·61)	0·35 (0·16–1·11)
Atropine methonitrate	0·63 (0·21–3·07)	0·60 (0·21–1·52)
Propantheline	0·59 (0·20–1·90)	1·97 (0·95–4·72)
Imipramine	Inactive (1–50)	
Amitriptyline	Inactive (1–50)	
Nortriptyline	Inactive (1–50)	
Amphetamine	Inactive (1–50)	
Phenelzine	Inactive (1–10)	
Chlorpromazine	Inactive (1–10)	
Perphenazine	Inactive (1–10)	
Haloperidol	Inactive (1–10)	
Phenobarbitone	Inactive (1–10)	
Methaqualone	Inactive (1–10)	
Phenytoin	Inactive (1–10)	
Chlordiazepoxide	Inactive (1–10)	
Codeine	Inactive (1–10)	
Morphine	Inactive (1–10)	

Drugs were administered intracerebroventricularly 1 h before intracerebroventricular injection of carbachol (2 μg/mouse) or oxotremorine (2 μg/mouse). Figures in parentheses indicate doses or dose ranges investigated.

route of administration are undoubtedly caused by actions within the brain, because these drugs were much less potent on systemic administration and their effects were inhibited by atropine-like substances injected intracerebroventricularly in doses much smaller than those which were effective on systemic administration. If these effects are mediated through central receptors which are normally activated by acetylcholine they may be 'muscarinic' effects not only because of their atropine sensitivity but also because they are not reproduced by intracerebroventricular nicotine.

The effects from activation of central receptors following the intracerebroventricular injection of the cholinomimetic drugs can be categorized as follows: (1) Salivation and lachrymation mediated through parasympathetic fibres and resulting from activation of parasympathetic nuclei in the brain. They can be blocked centrally or peripherally by atropine-like substances. (2) Hypothermia, due to intense vasodilatation in the skin and resulting from inhibition of vasoconstrictor tone. This explanation is consistent with the finding that the hypothermia following intracerebroventricular injection of cholinomimetic drugs is antagonized by systemic administration of a peripheral vasoconstrictor such as the α-adrenoceptor stimulant, oxymetazoline (unpublished observations). The central cholinergic receptors involved might reasonably be in the vasomotor centre and/or in the hypothalamic thermoregulatory centres. (3) Tremor, mediated through motor nerves and resulting from activation of extrapyramidal cholinergic mechanisms.

Pilocarpine and methacholine injected intracerebroventricularly are alike in being very active on parasympathetic nuclei and in having little effect on body temperature or tremor. They are distinctively different from carbachol and oxotremorine which resemble one another in causing parasympathetic effects, hypothermia and tremor. The possibility that these differences result from variations in the distribution of the drugs in the brain, or to their selective inactivation in particular parts of the brain, is unlikely because otherwise the quaternary compounds carbachol and methacholine would be expected to resemble each other in activity. An alternative explanation is that these drugs react in qualitatively different ways with particular atropine sensitive cholinoceptors in the brain. This explanation would require at least two kinds of such receptors, a result which would be of physiological importance if the receptors varied in their sensitivities to acetylcholine, the natural transmitter. There is, however, no evidence that they do so. The situation seems, therefore, to be similar to that involving selective stimulation of β-adrenoceptors. This phenomenon has been explained by supposing that 'unnatural' interactions occur between synthetic agonists, and β-adrenoceptors and their adjacent chemical groupings. The latter are presumed to vary and give rise to a family of unnatural receptors for unnatural agonists. These unnatural receptors have been called 'isoreceptors' (Jack, 1970; Brittain, Jack & Ritchie, 1970). The finding that oxotremorine and carbachol injected intracerebroventricularly cause tremor, hypothermia and parasympathomimetic effects and that pilocarpine and methacholine cause only the latter requires that there be at least two atropine sensitive isocholinoceptors in the brain. That oxotremorine causes greater tremor than carbachol may require a further isoreceptor.

It has been known for some time that atropine-sensitive cholinoceptors vary in their sensitivities to drugs. For example, bethanechol and carbachol are more active on the smooth muscle of the gastrointestinal tract and urinary bladder than

on vascular smooth muscle (Goodman & Gilman, 1970) whereas 1,4,5,6-tetrahydro-5-phenoxy-pyrimidine is a fairly potent muscarinic autonomic ganglion stimulant but is virtually inactive on heart muscle (Marshall, 1970). It is also known that gallamine (Riker & Wescoe, 1951) or pancuronium (Sexena & Bonta, 1970), selectively block cardiac muscarinic receptors. All these results are compatible with the concept that atropine sensitive cholinoceptors at the periphery exist in isoreceptor forms. The existence of isocholinoceptors would become of pharmacological and clinical importance if it should be possible to synthesize cholinomimetic drugs, or their antagonists, with selective effects on one or the other of these peripheral or central isoreceptors.

Although imipramine is known to have central anticholinomimetic activity (Cairncross, Gershon & Gust, 1963 ; Brimblecombe & Green, 1967 ; Tang & Schroeder, 1969) the tricyclic antidepressant drugs injected intracerebroventricularly did not antagonize the hypothermia caused by intracerebroventricular carbachol or oxotremorine. Perhaps the maximum tolerated dose of these drugs by this route was less than their effective intracerebroventricular anti-cholinomimetic dose. The oral efficacy of the tricyclic antidepressants in preventing hypothermia caused by intracerebroventricular carbachol or oxotremorine results chiefly from stimulation of heat production at the periphery, possibly by facilitating β-adrenoceptor effects, because their actions are blocked by β-adrenoceptor blocking agents but not by α-adrenoceptor blocking agents (Ankier, 1969). Systemic amphetamine appears to act in a similar way because its action is also prevented by β-adrenoceptor blockade but not by α-adrenoceptor blockade.

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