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Hypothermia following intraventricular injection of hemicholinium-3 in rats

P. SLATER

Department of Physiology, University of Manchester, Manchester 13

1. In unanaesthetized rats, a dose of 50 μg of hemicholinium-3 injected into the cerebral ventricles produced a prolonged hypothermia which was unaffected by the simultaneous administration of choline. This suggests that it was not due to inhibition of acetylcholine synthesis.

2. An intraperitoneal injection of 50 μg of hemicholinium-3 also produced hypothermia which, however, was blocked by choline which suggests that it is due to inhibition of acetylcholine synthesis.

3. Hyoscine and hexamethonium did not block the hypothermia produced by intraventricular hemicholinium-3, but some antagonism was obtained with phentolamine, imipramine and amphetamine.

4. When hemicholinium-3 was administered intraventricularly to rats pretreated with desmethylimipramine, a lethal hyperthermia developed.

5. It is concluded that no central cholinergic mechanism is involved in the hypothermia of the rat resulting from an intraventricular injection of hemicholinium-3.

Hemicholinium-3 is a potent inhibitor of acetylcholine synthesis both *in vitro* and *in vivo* (Gardiner, 1961 ; MacIntosh, 1963) and has proved to be a useful agent for investigating cholinergic mechanisms in the peripheral nervous system. Hemicholinium-3 does not penetrate the blood–brain barrier to any significant extent (Domer & Schueler, 1960), however, and consequently little is known about its effects on brain acetylcholine. Recent work has shown that the administration of hemicholinium-3 directly into the cerebral ventricles of both dogs and rats results in a considerable reduction in the amount of acetylcholine extractable from the brain tissue (Dren & Domino, 1968 ; Slater, 1968). If hemicholinium-3 were to be used as an inhibitor of acetylcholine synthesis in the central nervous system, it would be important to know whether it has any other central pharmacological actions.

A subject of some considerable interest at the present time is the hypothalamic regulation of body temperature and it has been proposed that the release of monoamines may be involved in this regulation (see Feldberg & Myers, 1964 ; Feldberg & Lotti, 1967). A cholinergic mechanism may be involved as well, for carbachol, oxotremorine and inhibitors of cholinesterase affect body temperature (Lomax & Jenden, 1966 ; Meeter & Wolthuis, 1968). A reduction in the amount of acetyl-

choline present may therefore have an effect on body temperature. This possibility has been investigated by administering hemicholinium-3 to rats and the results are presented in this paper.

Methods

Male Wistar rats weighing 200-250 g were used. Rectal temperatures were recorded either continuously or at intervals following drug administration.

Continuous temperature recording

Injections were made into the left lateral ventricle through an implanted cannula. The cannula consisted of a piece of stainless steel tubing (26 gauge) fixed with epoxy resin into a fine hole drilled through the centre of a piece of brass rod, 8 mm long and 2·5 mm diameter. The rod served both as a holder and a guide to the correct depth. The length of tubing which extended from the guide through the skull and into the ventricle was 3·5 mm. Under ether anaesthesia, the cannula was inserted vertically through a burr hole in the skull at a point 2·5 mm lateral and 1 mm posterior to the bregma. The stainless steel tube was connected by means of fine polythene tubing to a 50 μl. Hamilton syringe. The cannula was held in place with dental acrylic cement. A thermistor probe was inserted 6 cm into the rectum and taped to the tail. The animals were kept individually in plastic cages throughout the experiment. One hour after recovery from the ether, rectal temperature was plotted on a pen recorder for 5 min ; the rat was then injected from outside the cage and the temperature recorded continuously for 3 hr.

Temperature measurement at intervals

Groups of identical rats were used. The experiments were performed at a room temperature of 20°–21° C. Each animal was prepared for intraventricular injection under ether anaesthesia, the wound was infiltrated with a local anaesthetic and the rat was allowed to recover from the effects of the ether for 1 hr. Temperatures were recorded with a thermistor mounted in a rectal probe connected to a direct-reading thermometer. While the temperature was measured, each rat was restrained in a wire gauze cylinder, 8 cm diameter and 18 cm long, closed at one end. The animals were injected intraventricularly with 30 μl. of either isotonic drug solution or physiological saline solution and the rectal temperature was measured again at intervals after the injection.

Drugs

The following drugs were dissolved in pyrogen-free 0·9% NaCl solution and given by intraventricular injection: hemicholinium-3, triethylcholine chloride, choline chloride, hexamethonium bromide and bromolysergic acid. Drugs given by the intraperitoneal route were: hyoscine hydrobromide, propranolol, phentolamine, (+)-amphetamine sulphate, imipramine (Tofranil) and desmethylipramine (Pertofran).

Doses are expressed in terms of the salts and are given in the text.

Results

Hemicholinium-3

An intraventricular injection of 50 μg of hemicholinium-3 invariably caused a fall in rectal temperature. The extent of the hypothermia is shown in Fig. 1. Body temperature began to fall within 2 min of injection and continued to fall for 2 hr. An injection of 30 μl. of physiological saline solution resulted in a small increase in body temperature during the hour after the injection. A less pronounced fall in temperature was obtained with a dose of 5 μg of hemicholinium-3 (Fig. 2).

If hemicholinium-3 were to diffuse out of the central nervous system, the hypothermia could be due to a peripheral action. This possibility was investigated by

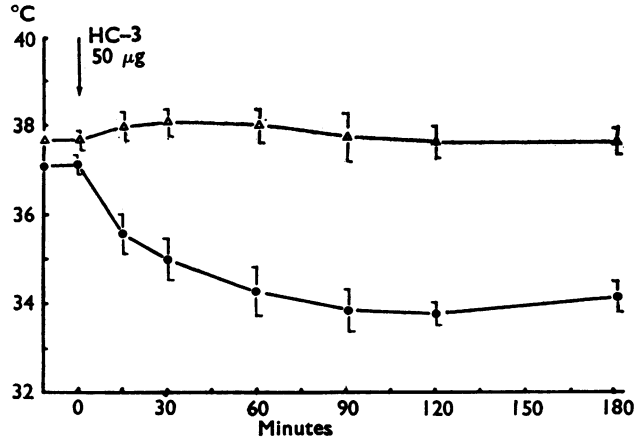

FIG. 1. Effect of hemicholinium-3 (50 μg intraventricularly) on the body temperature of the rat. Hemicholinium-3 (●——●) in four rats. Physiological saline (△——△) in four rats. Vertical bars indicate 2 × S.E. of the mean.

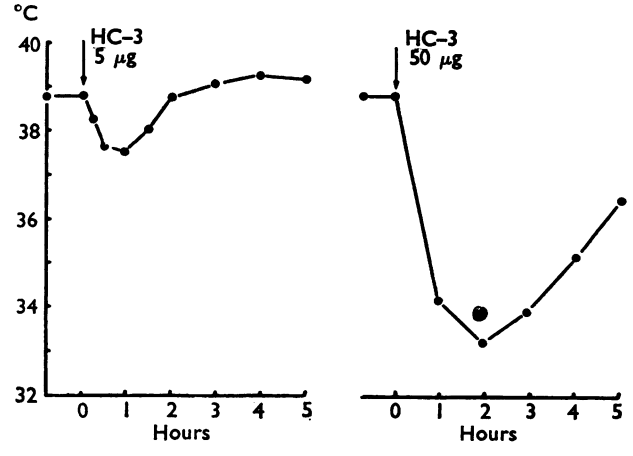

FIG. 2. Effect of hemicholinium-3 on body temperature of two rats. At the arrows, injection of 5 μg and 50 μg into the cerebral ventricles.

administering choline, which prevents the depletion of acetylcholine produced by hemicholinium-3. The results are summarized in Table 1. An intraperitoneal injection of 50 μg of hemicholinium-3 produced a fall in body temperature, which up to 40 min after injection was similar to the fall in temperature resulting from an intraventricular injection of the same amount of hemicholinium-3. All the animals which received hemicholinium-3 by the intraperitoneal route, however, were dead within 1 hr. When hemicholinium-3 and choline were given together by the intraperitoneal route, the fall in body temperature was prevented. On the other hand, choline given either intraperitoneally or intraventricularly had no effect on the fall in body temperature resulting from an intraventricular injection of hemicholinium-3.

Triethylcholine

Like hemicholinium-3, an injection of triethylcholine into the cerebral ventricles of rats reduces the acetylcholine content of the brain (Slater, 1968). In Fig. 3 the effect on temperature of 50 μg hemicholinium-3 injected intraventricularly into one rat is compared with that of 200 μg triethylcholine similarly injected into another rat. Triethylcholine produced a fall in body temperature but to a much less extent and of shorter duration than that resulting from the injection of hemicholinium-3.

TABLE 1. *Effects of hemicholinium-3 and choline on the body temperature of the rat*

Compounds administered	Route of administration	Dose	Change in body temperature (°C) at 20, 40 and 60 min after injection		
			20 min	40 min	60 min
Hemicholinium-3	Intraventricular	50 μg	−3·0±0·3	−3·8±0·5	−4·2±0·4
Choline	Intraventricular	600 μg	−1·3±0·3	−1·2±0·2	−0·8±0·2
Hemicholinium-3	Intraventricular	50 μg	−2·3±0·4	−3·4±0·6	−4·0±0·6
+choline	Intraventricular	50 mg/kg			
Hemicholinium-3	Intraperitoneal	50 μg	−3·1±0·4	−4·5±0·5	—
Choline	Intraperitoneal	50 mg/kg	−0·3±0·1	−0·7±0·1	−1·1±0·2
Hemicholinium-3	Intraperitoneal	50 μg	−0·6±0·1	−0·8±0·2	−1·4±0·2
+choline	Intraperitoneal	50 mg/kg			
Hemicholinium-3	Intraventricular	50 μg	−4·2±0·4	−4·7±0·4	−4·5±0·5
+choline	Intraperitoneal	50 mg/kg			

Each result is the mean±S.E. obtained from four animals.

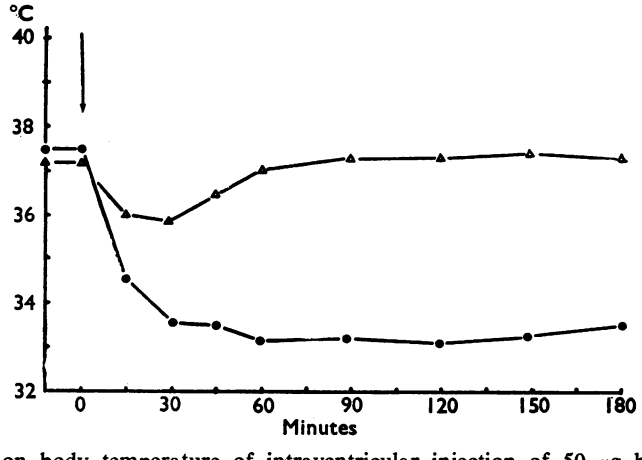

FIG. 3. Effects on body temperature of intraventricular injection of 50 μg hemicholinium-3 (●——●) into one, and of 200 μg triethylcholine (△——△) into another rat.

TABLE 2. *Effects of a number of drugs on hemicholinium-3(HC-3)-induced hypothermia in rats*

Drugs	Dose and route of injection of the test drug		Change in body temperature (°C) at 20, 40 and 60 min after hemicholinium-3 or after the test drug when given alone		
			20	40	60
HC-3	—	—	−2·5±0·3	−3·1±0·3	−3·7±0·3
Hyoscine+HC-3	20 mg/kg	Intraperitoneal	−2·6±0·4	−3·5±0·2	−3·6±0·3
Hexamethonium	200 μg	Intraventricular	−2·2±0·1	−2·1±0·2	−1·8±0·1
Hexamethonium+HC-3	200 μg	Intraventricular	−3·0±0·5	−5·0±0·6	−5·4±0·4
Bromolysergic acid+HC-3	30 μg	Intraventricular	−1·8±0·1	−2·8±0·2	−3·9±0·2
Propranolol+HC-3	250 μg	Intraventricular	−2·1±0·3	−2·7±0·4	−3·1±0·5
Phentolamine	100 μg	Intraventricular	+0·4±0·1	+0·4±0·1	+0·3±0·1
Phentolamine+HC-3	100 μg	Intraventricular	−1·4±0·4	−2·0±0·4	−2·4±0·3
(+)-Amphetamine	5 mg/kg	Intraperitoneal	+0·9±0·3	+1·7±0·4	+1·8±0·3
(+)-Amphetamine+HC-3	5 mg/kg	Intraperitoneal	+0·1±0·02	+0·1±0·02	+0·3±0·04
Imipramine	15 mg/kg	Intraperitoneal	+0·3±0·01	+0·2±0·01	+0·5±0·01
Imipramine+HC-3	15 mg/kg	Intraperitoneal	−0·8±0·2	−1·1±0·2	−0·3±0·2

The drugs were administered 60 min before an intraventricular injection of hemicholinium-3 (50 μg). Each result represents the mean±S.E. obtained with four animals. Where the test drug alone affected body temperature, the result is also shown.

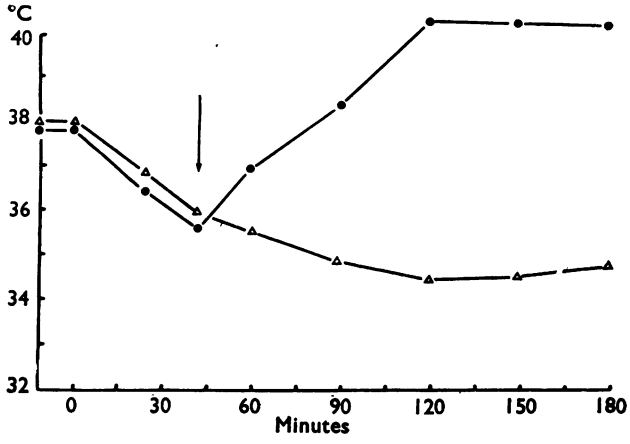

FIG. 4. Effect of (+)-amphetamine on hemicholinium-3 hypothermia. Both rats were injected with 50 μg hemicholinium-3 intraventricularly; at the arrow one animal (●——●) received 5 mg/kg (+)-amphetamine.

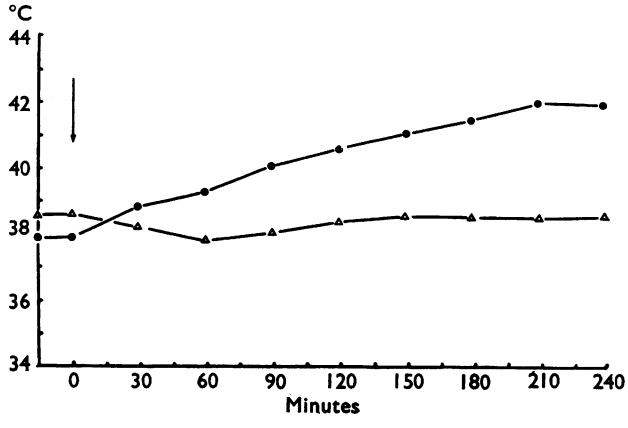

FIG. 5. Effect of desmethylimipramine on hemicholinium-3 hypothermia. At the arrow one rat (△——△) received desmethylimipramine 50 mg/kg intraperitoneally, and the other (●——●) hemicholinium-3 50 μg intraventricularly. This rat had received 1 hr earlier desmethylimipramine 50 mg/kg intraperitoneally.

Effects of drugs on hemicholinium-3-induced hypothermia

Groups of four rats were pretreated with one of a number of pharmacological agents, followed 1 hr later by 50 μg of hemicholinium-3 injected into the cerebral ventricles. Body temperature was measured 5 min before the administration of hemicholinium-3 and 20, 40 and 60 min afterwards. The mean changes in body temperature recorded in each group are shown in Table 2. When the drug alone affected body temperature the results are shown in Table 2 as well. Pretreatment with intraperitoneal hyoscine had no effect on hemicholinium-3-induced hypothermia. Hexamethonium itself reduced body temperature when injected into the cerebral ventricles and when injected together with hemicholinium-3, the effects of the two were additive. Phentolamine, but not propranolol, partially blocked the hypothermia after hemicholinium-3. The injection of propranolol alone did not affect body temperature. An intraventricular injection of bromolysergic acid had no effect on body temperature, neither did it affect hemicholinium-3-induced hypothermia. An intraperitoneal injection of amphetamine alone caused a rise in temperature which was almost completely prevented when followed by an intraventricular injection of hemicholinium-3. Further, as shown in Fig. 4, once the hemicholinium-3-induced hypothermia had occurred it was effectively reversed by amphetamine.

The antidepressant imipramine, which by itself had a weak hyperthermic effect, greatly reduced the hypothermic action of a subsequent intraventricular injection of hemicholinium-3. In preliminary experiments it was found that desmethylimipramine which by itself did not affect body temperature converted the hemicholinium-3-induced hypothermia into a long lasting hyperthermia. This is illustrated in Fig. 5 which shows the effect of desmethylipramine alone and, in another rat, the effect of the hemicholinium-3 given 1 hr after the desmethylipramine. Body temperature, which was recorded continuously reached 42° C after which the rat died.

Discussion

The central actions of hemicholinium-3 so far reported are e.e.g. seizures in the dog (Shellenberger & Domino, 1967 ; Dren & Domino, 1968) and a reduction in brain acetylcholine (Slater, 1968). The present experiments have brought to light another central action of hemicholinium-3. Injected into the cerebral ventricles of rats, it produced long lasting hypothermia. The question arises whether the three effects are related to one another.

Hemicholinium-3 is not the only drug which reduces brain acetylcholine. The same effect occurs when triethylcholine is injected into the cerebral ventricles of rats (Slater, 1968). The present experiments show that such injections also lower body temperature although the effect is not as strong as that of hemicholinium-3, and is of much shorter duration. Yet the depletion of the brain acetylcholine also did not last as long as after hemicholinium. In fact the time courses of the hypothermias produced by the two compounds parallel the time courses previously found for the reduction in brain acetylcholine. This suggests that acetylcholine, or lack of acetylcholine, may be responsible for the hypothermia. There are other findings which suggest that, at least in the rat, acetylcholine is involved in the regulation of body temperature. Kirkpatrick & Lomax (1967) produced hypothermia in the rat with atropine injected either systemically or into regions of the hypothalamus, and

Meether & Wolthuis (1968) found that in rats, but not in other species, inhibitors of cholinesterase produced hypothermia, an effect which was partially blocked by atropine.

The hypothermia resulting from an intraventricular injection of hemicholinium-3, however, appears not to be due to the reduction in brain acetylcholine. This reduction was found to be prevented by intraventricular injections of choline (Slater, 1968) whereas the hypothermia obtained in the present experiments with intraventricular hemicholinium-3 was not affected by choline injected either intraventricularly or intraperitoneally. It is also unlikely that the hypothermia results from an action on acetylcholine receptors in the central nervous system because it was not affected by hyoscine.

Hemicholinium-3 also produced hypothermia when injected intraperitoneally but this hypothermia was prevented by choline. It is therefore probably due to the inhibition of acetylcholine synthesis and may be brought about by impairment of the mechanisms for heat production because the ensuing block of neuromuscular transmission would result in a loss of muscle tone.

Not only the reduction in brain acetylcholine, but also the e.e.g. seizures seem to be unrelated to the hypothermia. Such seizures are also produced in rats by intraventricular injections of hemicholinium, but unlike the hypothermia they are prevented by hexamethonium (unpublished experiments).

The work of Feldberg and others has implicated catecholamines and 5-hydroxytryptamine as being involved in the central regulation of body temperature. In the rat, adrenaline and noradrenaline given intraventricularly produce hyper- or hypothermia depending on the dose (Feldberg & Lotti, 1967). The finding that the sympathomimetics amphetamine, imipramine and desmethylimipramine antagonize hemicholinium-3-induced hypothermia may signify that hemicholinium-3 affects the release or metabolism of catecholamines in the central nervous system.

Why desmethylimipramine should reverse the hypothermia is not clear; it has a similar effect in mice treated with oxotremorine (Morpurgo, 1967).

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