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Inhibition by atropine of the increased turnover of noradrenaline in the hypothalamus of rats exposed to cold

M. A. SIMMONDS*

Department of Pharmacology, University of Cambridge

Summary

1. Small doses of (−)-[^{3}H] noradrenaline were injected into the lateral cerebral ventricles in rats to label radioactively the endogenous noradrenaline (NA) stores.

2. Intraventricular injection of 25 μg atropine methonitrate at the same time inhibited the increased rate of disappearance of [^{3}H] NA from the hypothalamus at an environmental temperature of 9° C, when compared with the values at 24° C, without impairing temperature regulation.

3. At 32° C, 25 μg atropine methonitrate caused a lethal hyperthermia. A dose of 5 μg was not lethal and did not inhibit the increased rate of disappearance of [^{3}H] NA from the hypothalamus.

4. It is concluded that the pathway which stimulates an increased turnover of NA in the cold contains an atropine sensitive synapse but is not the principal pathway of heat production. The increased turnover of NA in the heat probably does not involve an atropine sensitive synapse.

Introduction

Exposure of rats to heat or cold increases the turnover of noradrenaline (NA) in whole brain (Costa & Neff, 1966; Gordon, Spector, Sjoerdsma & Udenfriend, 1966; Corrodi, Fuxe & Hökfelt, 1967; Duce, Crabai, Vargiu, Piras, Adamo & Gessa, 1968; Reid, Volicer, Smookler, Beaven & Brodie, 1968). These responses occur specifically in the hypothalamus of the rat upon exposure to mild heat or cold (Simmonds, 1969).

There is also evidence that cholinergic substances may be involved in the central regulation of body temperature. The injection of atropine into the rostral hypothalamus of the rat causes a rise in rectal temperature (Kirkpatrick & Lomax, 1967) and the injection of acetylcholine into the same area causes a fall in temperature which is antagonized by atropine (Beckman & Carlisle, 1969; Kirkpatrick & Lomax, 1970). Anticholinesterase drugs similarly cause a fall in rectal temperature through an action in the brain (Meeter & Wolthuis, 1968; Friedman & Jaffe, 1969). These responses are not always reproduced, however, since Myers & Yaksh (1968) have reported that intraventricularly injected acetylcholine and eserine cause a rise in the temperature of the rat.

* Present address: Department of Pharmacology, The School of Pharmacy, University of London, 29/39 Brunswick Square, London, WC1N 1AX.

Interactions between adrenergic and cholinergic pathways in the brain have been described. Lomax, Foster & Kirkpatrick (1969) found that the injection of NA into the rostral hypothalamus of the rat blocked the hypothermic response to pilocarpine, while Bhagat, Kramer & Seifter (1967) showed that an intraventricular injection of acetylcholine increased the rate of disappearance of [^{3}H] NA from the rat brain.

It was of interest, therefore, to determine whether there were atropine-sensitive steps in the pathways leading to the increased rate of disappearance of [^{3}H] NA from the hypothalamus of rats exposed to heat or cold (Simmonds, 1969).

Methods

Male Wistar rats (200–250 g) were kept in the laboratory at an environmental temperature of 21–25° C for at least 18 h before the start of an experiment. The day before each experiment, the rats to be used were anaesthetized with ether and a hole was drilled through the skull according to the method of Noble, Wurtman & Axelrod (1967). The rats were then allowed to recover. This enabled injection into the lateral ventricle on the next day to be carried out under light ether anaesthesia with the minimum of surgical interference.

Each rat was injected intraventricularly with 2·5 μCi (0·194 μg) of (−)-7-[^{3}H] noradrenaline (Radiochemical Centre, Amersham, Bucks) in 20 μl artificial cerebrospinal fluid (Merlis, 1940) containing 0·001% ethylenediaminetetra-acetic acid and 0·002% ascorbic acid at pH 6·0–6·5. Some rats received 25 μg or 5 μg atropine methonitrate in the same injection. After 30 min at room temperature, the rats were placed in individual cages in an environment of 9, 24 or 32° C controlled to within ±2° C. Two and 6 h after injection, the rats were stunned and decapitated immediately after their temperatures had been measured with a Grant Thermistor probe inserted 5 cm into the rectum. The brains were removed and handled as previously described (Simmonds, 1969). The cerebellum and medulla were discarded and the hypothalamus was dissected from the 'rest of brain'.

[^{3}H] NA in the hypothalamus and 'rest of brain' was extracted into perchloric acid, isolated on a strong cation exchange resin and estimated by liquid scintillation counting as previously described (Simmonds, 1969). The fall in concentration of [^{3}H] NA in each area of brain 2–6 h after injection was expressed as a percentage of the concentration at 2 h after injection. Values of the percentage of [^{3}H] NA disappearing from the brain during this time are analogous to estimates of fractional rate constant which are calculated when the concentration of [^{3}H] NA has been measured at more than two points in time.

Results

Disappearance of [^{3}H] NA from control rats

In the hypothalamus of control rats, which did not receive atropine, the retention of [^{3}H] NA at 2 h after injection was similar at 9 and 24° C (Table 1). At 32° C, however, the retention was significantly reduced. A similar pattern of results was seen in the 'rest of brain' (Table 1). Two–6 h after injection, the rate of disappearance of [^{3}H] NA was significantly greater ($P<0·05$) at an environmental temperature of 9° C than at 24° C (Fig. 1). The disappearance of [^{3}H] NA at 32° C was also greater than at 24° C, the difference approaching significance ($P<0·1>$

0·05). In the ' rest of brain ', environmental temperature had no effect on the rate of disappearance of [^{3}H] NA.

The rectal temperatures of control rats were significantly elevated by 0·5° C ($P<0·05$) at an environmental temperature of 9° C and by 2·1–2·7° C ($P<0·05$) at 32° C when compared with the values at 24° C (Table 2).

Disappearance of [^{3}H] NA from atropine treated rats

Environmental temperature 24° C

Atropine methonitrate (25 μg) injected simultaneously with the [^{3}H] NA had no effect on the retention of [^{3}H] NA by either hypothalamus or ' rest of brain ' at 2 h (Table 1). Atropine also had no effect on the rate of disappearance of [^{3}H] NA from either brain area between 2 and 6 h (Fig. 1). The rectal temperatures of the atropine treated rats were slightly higher than controls (Table 2) but the differences were not significant.

Environmental temperature 9° C

An intraventricular dose of atropine methonitrate (25 μg) caused a small reduction in the retention of [^{3}H] NA by the hypothalamus at 2 h after injection (Table 1), but the reduction was not quite significant ($P<0·1>0·05$). There was little change in the retention of [^{3}H] NA by the ' rest of brain ' at 2 h in the presence of atropine. The rate of disappearance of [^{3}H] NA from the hypothalamus between 2 and 6 h, however, was significantly reduced in atropine treated rats when compared with controls (Fig. 1), while the simultaneous disappearance of [^{3}H] NA from the ' rest

TABLE 1. *Concentration of [^{3}H] NA in rat brain tissues 2 h after intraventricular injection of (–)-[^{3}H] NA (2·5 μCi) with and without atropine*

	Hypothalamus			Rest of brain		
Environmental temperature (°C)	9	24	32	9	24	32
Control	385±23 (7)	333±21 (6)	176±11* (9)	95·4±6·5 (7)	87·0±4·9 (6)	46·8±2·7* (9)
Atropine	302±33 (9)	291±27 (6)	257±26 (9)	87·5±5·8 (9)	93·1±7·7 (6)	62·0±6·4† (9)
Significance of difference from control	$P<0·1$ $<0·05$		$P<0·05$			$P<0·05$

† $P<0·01$, * $P<0·001$ when compared with the value at 24° C. Dose of atropine methonitrate was 25 μg/rat intraventricularly at 9 and 24° C and 5 μg/rat intraventricularly at 32° C. Each value is mean±S.E. (pCi/mg tissue) of the number of results shown.

TABLE 2. *Rectal temperatures (°C) of rats under different environmental conditions 2 and 6 h after the injection of (–)-[^{3}H] NA with and without atropine*

Environmental temperature	9° C		24° C		32° C	
Time after injection	2 h	6 h	2 h	6 h	2 h	6 h
Control	38·4±0·10 (7)	38·5±0·10 (9)	37·9±0·08 (5)	38·0±0·10 (6)	40·0±0·18 (9)	40·7±0·13 (8)
Atropine	38·7±0·08 (9)	38·7±0·13 (7)	38·2±0·14 (6)	38·3±0·14 (5)	40·4±0·12 (9)	41·1±0·34 (8)

Dose of atropine methonitrate was 25 μg/rat intraventricularly at 9 and 24° C and 5 μg/rat intraventricularly at 32° C. Each value is mean±S.E. of the number of results shown.

of brain ' was not significantly affected by atropine. The rectal temperatures of the rats were slightly elevated by atropine, the difference at 2 h being significant ($P<0{\cdot}05$) (Table 2).

Environmental temperature 32° C

Exposure to 32° C following an intraventricular dose of 25 μg atropine methonitrate resulted in most of the rats dying in severe hyperthermia (rectal temperatures in excess of 42·4° C) within 6 hours. The dose was, therefore, reduced to 5 μg at which the mortality rate was less than 15% within 6 h of injection. This latter dose of atropine methonitrate caused a significant increase ($P<0{\cdot}05$) in the retention of [^{3}H] NA by both hypothalamus and ' rest of brain ' at 2 h after injection (Table 1). The rate of disappearance of [^{3}H] NA 2–6 h after injection, however, was also significantly increased by atropine in both hypothalamus and ' rest of brain ' (Fig. 1). The rectal temperatures of the atropine treated rats were slightly, but not significantly, higher than those of controls at 32° C (Table 2).

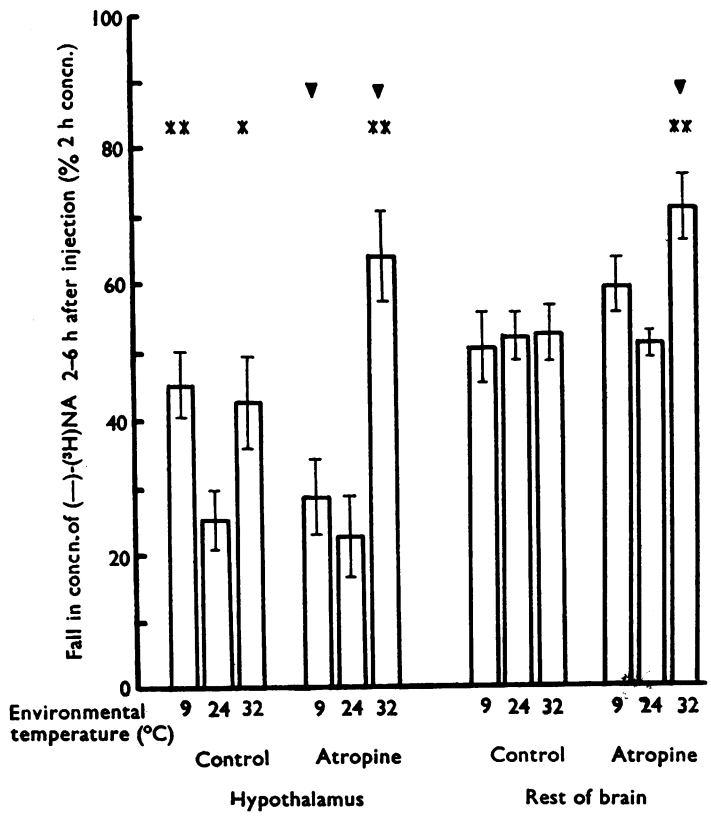

FIG. 1. Fall in concentration of [^{3}H] NA in the hypothalamus and ' rest of brain ' of rats 2–6 h after intraventricular injection of [^{3}H] NA. Results are expressed as a percentage of the concentration 2 h after injection and are shown as mean ± S.E. of five to nine values. Atropine treated rats received an intraventricular dose of atropine methonitrate at the same time as the [^{3}H] NA. The dose was 25 μg in experiments at 9 and 24° C, and 5 μg in experiments at 32° C. Rats were exposed to the environmental temperatures shown from 30 min after injection. ▼, Significantly different from corresponding control value ($P<0{\cdot}05$). **, Significantly different from adjacent 24° value ($P<0{\cdot}05$). *, Difference from adjacent 24° value approaching significance ($P<0{\cdot}1>0{\cdot}05$).

Discussion

The basic design of these experiments differs in two ways from that used in an earlier study (Simmonds, 1969). (−)-[^{3}H] NA has been injected instead of (±)-[^{3}H] NA and the injections have been made by the intraventricular instead of the intracisternal route. Nevertheless, the results obtained from control rats in this study confirm those obtained in earlier experiments, the only difference being that the increases in the rate of disappearance of [^{3}H] NA at 9 and 32° C are not as great as before. This may be due to differences in the distribution of [^{3}H] NA following injection by different routes (Iversen & Simmonds, 1969). It is unlikely, however, that the use of the laevo isomer in these experiments had much advantage over the use of racemic [^{3}H] NA since there is evidence that (−)-[^{3}H] NA and (+)-[^{14}C] NA disappear at the same rate from rat brain (Iversen & Simmonds, unpublished results).

In control rats at 32° C, the retention of [^{3}H] NA at 2 h after injection was significantly less than in rats exposed to lower environmental temperatures. This reduction was seen in both hypothalamus and 'rest of brain' and in both cases was antagonized by atropine. Since these effects occurred in the brain as a whole and showed little relationship to the subsequent rates of disappearance of [^{3}H] NA, the retention of [^{3}H] NA at 2 h after injection may be largely determined by other factors such as blood flow through the brain.

Injection of atropine methonitrate (25 μg) into the c.s.f. of rats exposed to 9° C did not impair temperature regulation, whereas the same dose injected into rats at 32° C resulted in a lethal hyperthermia. This is considered to be a central action of atropine since the quaternary form of atropine used should not easily pass out of the brain. In addition, peripherally injected atropine causes hypothermia rather than hyperthermia (Kirkpatrick & Lomax, 1967). It would appear, therefore, that there is an atropine sensitive synapse in the central heat loss pathway in the rat but not in the heat production pathway. Although atropine may not block the heat production pathway, it does block the increased rate of disappearance of [^{3}H] NA from the hypothalamus in the cold. This suggests that the pathway involving an atropine-sensitive synapse and an increased turnover of NA in the cold is not the principal pathway of heat production. Instead, it could be an inhibitory pathway which reduces heat loss. Such a scheme has previously been proposed by Bligh & Maskrey (1969) for the sheep, although this species differs from the rat in that it appears to have a cholinergic synapse in the main pathway of heat production. The idea that NA may not be involved in the principal pathway of heat production in the rat is also supported by evidence that degeneration of NA-containing nerve terminals in the rat brain following injection of 6-hydroxydopamine does not impair temperature regulation (Simmonds & Uretsky, 1970).

The presence of an atropine sensitive synapse in the principal heat loss pathway in the rat required that the dose of atropine be reduced if the animals were to survive in the heat. The subsequent failure to detect an atropine sensitive synapse in the pathway leading to an increased turnover of NA in the heat must, therefore, be interpreted with caution, since the dose of atropine may have been too low. There was, however, sufficient atropine present to cause a significant increase in the rate of disappearance of [^{3}H] NA from both the hypothalamus and the 'rest of brain' at 32° C. This latter response may be related to the possible development of a stress situation following the partial impairment of the heat loss mechanism by atropine. Similar increases in the turnover of NA throughout the brain have

been reported to occur during other stressful situations (Thierry, Javoy, Glowinski & Kety, 1968).

The specific increase in the turnover of NA in the hypothalamus of rats exposed to cold is confined to an area which is at present indistinguishable from that in which heat exposure causes a similar increase in NA turnover (Simmonds, 1969). Both increases could be detected in anterior and posterior parts of the hypothalamus but not in the preoptic area. These results, however, suggest that there are differences in the pathways which lead to the increased turnover of NA in heat and cold. The pathway which is stimulated in the cold contains an atropine-sensitive synapse whereas the pathway stimulated in the heat probably does not.

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