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Role of taurine as a possible transmitter in the thermoregulatory pathways of the rat

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Taurine (10 and 20 μg) injected unilaterally into the lateral ventricle of rats caused an increase in core temperature. Bilateral injection of taurine 2·5 and 5 μg into the preoptic region of the anterior hypothalamus induced a dose-related hyperthermia: higher doses (10 μg) caused hypothermia. Intrahypothalamically taurine-induced hyperthermia was blocked by prior injection of strychnine hydrochloride (5 and 15 μg); doses which alone had no effect on core temperature. Of the other inhibitory amino acids injected intrahypothalamically hypotaurine also induced a hyperthermia. GABA (10 μg) caused hypothermia; glycine (10 μg) had no effect. Potassium (50 mM) stimulated release of radioactivity from superfused slices of anterior hypothalamus prelabelled with [^{3}H]taurine in a calcium-dependent manner. A high affinity uptake mechanism with a K_m of 8·5 μM was demonstrated with [^{3}H]taurine into slices of anterior hypothalamus. Taurine may have a neurotransmitter role in the anterior hypothalamus but whether the body temperature effects represent physiological or pharmacological events remains to be established.

Many putative neurotransmitters are widely accepted as having a physiological role in temperature regulation. Relatively little, however, is known about the actions of amino acid transmitters on body temperature (for review see Cox & Lomax 1977).

There is strong evidence to suggest that taurine (2-aminoethanesulphonic acid) is an inhibitory neurotransmitter amino acid in the mammalian central nervous system (c.n.s.) (Davison & Kaczmarek 1971; Barbeau et al 1975; Johnston 1975). Taurine has been reported to have a heterogenous distribution within the c.n.s. with high concentrations in the hypothalamus, striatum, cerebellum and retina (Bonaventure et al 1974; Barbeau et al 1975). Similarly high levels of the taurine synthesizing enzymes, cysteine oxidase and cysteine sulphinate decarboxylase, have also been demonstrated within these brain regions (Pasantes-Morales et al 1977). The amino acid is associated with subcellular fractions enriched with synaptic vesicles (De Belleroche & Bradford 1973) and neuronal tissue possesses a high-affinity uptake system for taurine (Sieghart & Karobath 1974; Hruska et al 1978). In addition, depolarizing stimuli can evoke release of radiolabelled taurine from rat brain slices in a calcium-dependent fashion (Kaczmarek & Davison 1972) and in vivo release of [^{14}C]taurine from cat cerebral cortex has been demonstrated (Kaczmarek & Adey 1974).

In keeping with its hypothalamic location, several roles for taurine have been proposed including that of temperature regulation (Hruska et al 1973). However, this study involved use of high doses of taurine administered peripherally to mice. We have, therefore, investigated the effect of centrally-administered taurine in rats, in order to define more precisely the effect of this amino acid on body temperature. To test the hypothesis that taurine may be a hypothalamic transmitter substance we have studied the uptake into and release of [^{3}H]taurine from slices of rat hypothalamus in vitro.

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METHODS

Preparation of animals and intracerebral injections

Male Porton rats (250–300 g) were used. Stainless steel guide cannulae (outer diameter 0·65 mm) were implanted into the brains of rats anaesthetized with chloral hydrate (300 mg kg^{-1}, i.p.), so that the tip of the guide cannulae lay 2 mm above the desired injection site. Target areas for focal injections were the preoptic region of the anterior hypothalamus (A + 7·0, L ± 2·5, V−2·3) and the striatum (A + 7·0, L ± 3·0, V 0) (König & Klippel 1963). One week later, an injection cannula whose tip lay 2 mm beyond the end of the guide cannula was used for drug injection. Taurine (free acid—Sigma) or saline (pH 6·8 or 7·4) was injected unilaterally into the lateral ventricle (i.c.v.) or bilaterally into the preoptic hypothalamus (i.h.) or striatum (i.s.). In other experiments hypotaurine (Calbiochem), glycine (Fisons) or γ-amino-n-butyric acid (GABA) (Sigma) were injected bilaterally into the preoptic hypothalamus. All agents were injected in a volume of 1 μl. The vehicle used was 0·9% sterile pyrogen-free NaCl

(saline) (pH 7·4) injected in a volume of 1 μl. In addition, saline, adjusted to pH 6·8 to more closely mimic the concentrated taurine solutions, was also used as injection controls. After completion of an experiment the position of the cannula tract was verified by macroscopic examination.

In order to estimate the possible spread of drug after intracerebral administration, [^{3}H]taurine in 1 μl (sp. act. 9 Ci mM^{-1}) was injected into one side of the preoptic hypothalamus. Rats were killed after 10 min, the forebrain region dissected out and cut into 1 mm × 2 mm slices, and the radioactivity content of each slice determined by liquid scintillation counting.

In one series of experiments strychnine hydrochloride (Sigma) (5 ng–15 μg in 1 μl saline) was injected bilaterally intrahypothalamically followed immediately by an injection of taurine (5 μg in 1 μl saline) or saline.

Temperature measurements

Core temperature was measured in conscious lightly-restrained animals with rectal thermistor probes (Light Labs.), inserted to a depth of 4 cm. Unless stated otherwise temperature was always recorded at an ambient temperature 22 ± 0·5 °C, and rats were acclimatized at this temperature for at least 2 h before the experiment.

Statistics

Comparisons of body temperature of drug-injected and control (saline-injected) groups were made using the non-parametric Mann-Whitney U test and unless otherwise stated a statistically significant difference between groups was taken as $P < 0{\cdot}05$.

Release and uptake studies

Release of labelled amino acid from brain slices has been described elsewhere (Srnivisan et al 1969). Briefly, anterior hypothalamus from freshly dissected rat brain was chopped in two directions at 0·2 mm intervals on a McIlwain tissue chopper and pre-incubated at 37 °C for 20 min in 5 ml of Krebs bicarbonate buffer (pH 7·4) containing 2 μCi ml^{-1} of [^{3}H]taurine (9·0 Ci mM^{-1}; Radiochemical Centre, Amersham). The slices were then rinsed and transferred to small plexiglass cylinders and superfused with Krebs bicarbonate buffer at a rate of 1·0 ml min^{-1}. After an initial washout period of 30 min, fractions were collected every 2 min for determination of radioactive content. Six min after the start of collection, the superfusing medium was changed to one containing 50 mM KCl for 4 min. In studies with low calcium concentration in the superfusing medium $MgCl_2$ was substituted for $CaCl_2$. At the end of the superfusion tissue was recovered, solubilized and radioactive content determined. Results are expressed as rate constants, representing the per cent of total tissue radioactivity released into the medium per minute.

In another series of experiments the uptake of [^{3}H]taurine into slices of anterior hypothalamus was studied. Tissue slices (5 mg) were preincubated for 10 min in 2 ml Krebs-Ringer bicarbonate buffer at 37 °C. [^{3}H]Taurine was added to each tube (final concentration 0·5–5 μM) and the samples were incubated for 5 min. Uptake was terminated by rapid filtration of each sample on Whatman glass fibre filters. Following washing with 12 ml ice-cold buffer, radioactivity in each tissue sample was determined by liquid scintillation counting. Tissue blanks were determined by measuring the uptake of radioactivity into tissue slices incubated in Krebs in an ice bath at 0 °C. The means of quadruplicate determinations (s.e. of means $< 10\%$) were fitted to double reciprocal Lineweaver-Burke plots by the method of least squares. The velocity of [^{3}H]taurine uptake was expressed as mol g^{-1} h^{-1}.

RESULTS

Taurine and body temperature

Centrally administered taurine (10 and 20 μg, i.c.v.) produced a significant increase in body temperature (+1·8 °C at 20 min for the 10 μg dose, $P < 0{\cdot}05$) (Fig. 1a). Saline (pH 6·8 or 7·4) or 5 μg taurine had no effect on core temperature by this route. The 10 μg dose of taurine had a more effective and prolonged action than the 20 μg dose administered into the lateral ventricle (Fig. 1a).

Bilateral intrahypothalamic injection of 2·5 and 5 μg taurine resulted in significant hyperthermia (+1·3 °C at 20 min, $P < 0{\cdot}05$), while 10 μg taurine (i.h.) produced a significant hypothermia (−0·9 °C at 15 min, $P < 0{\cdot}05$). (Fig. 1b). The effect of i.h. taurine on body temperature lasted 15–20 min. Neither saline (pH 6·8) nor taurine (10 and 20 μg) produced significant changes in body temperature when injected bilaterally into the striatum. The areas of the injection sites for intrahypothalamic and intrastriatal administration of taurine are shown in Fig. 2. The pattern of spread of radioactivity 10 min following focal injection of [^{3}H]taurine into the anterior hypothalamus shows that the amino acid diffuses approximately 1–2 mm away from the target site within this time (Fig. 2).

Pretreatment with bilateral intrahypothalamic

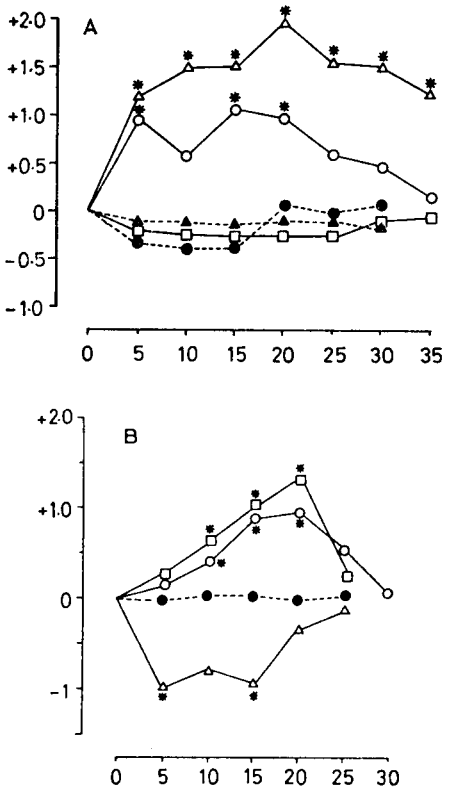

FIG. 1A. Time course of the core temperature (ordinate: △ °C) response to i.c.v. taurine at doses of 5 μg (□), 10 μg (△) and 20 μg (○). (Closed symbols denote i.c.v. saline—pH 6·8 (●) and pH 7·4 (▲)). Abscissa: time (min).
B. Time course of the core temperature (ordinate: △ °C) response to bilateral intrahypothalamic taurine at doses of 2·5 μg (○), 5 μg (□) and 10 μg (△). (● denotes i.h. saline adjusted to pH 6·8). Abscissa: time (min).

strychnine, in doses of 5 and 15 μg, inhibited the hyperthermic response evoked by 5 μg taurine (i.h.) in a dose-related manner (Fig. 3). Lower doses of strychnine (5 ng and 0·5 μg) were ineffective at blocking taurine-induced hyperthermia. None of these doses of strychnine when injected bilaterally into the hypothalamus alone had any significant effect on the core temperature of rats at an ambient temperature of 22 °C.

Other inhibitory amino acids and body temperature

In a similar manner to taurine, its precursor hypotaurine (10 and 20 μg) produced a hyperthermic effect when injected bilaterally into the preoptic region of the anterior hypothalamus. The largest increases were associated with the 10 μg dose, a plateau being seen between 15–30 min (+1·1 ± 0·2 °C at 30 min,

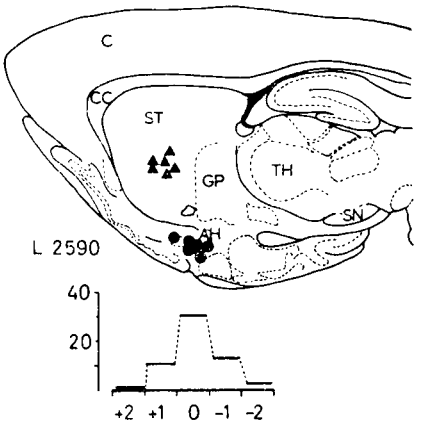

FIG. 2. Longitudinal section through the rat brain taken from the plane L2590 μ of the atlas of König & Klippel (1963) to illustrate positions of injection sites within the preoptic region of the anterior hypothalamus (●) and the striatum (▲). The lower tracing shows the relative diffusion of radioactivity from target site 10 min after injection of 1 μl [^{3}H]taurine solution into the anterior hypothalamus. (Total radioactivity recovered was 60% of that injected). Abbreviations used: AH, anterior hypothalamus; C, cortex; CC, corpus callosum; GP, globus pallidus; SN, substantia nigra; ST, striatum; TH, thalamus. Ordinate: radioactivity (%). Abscissa: time (min).

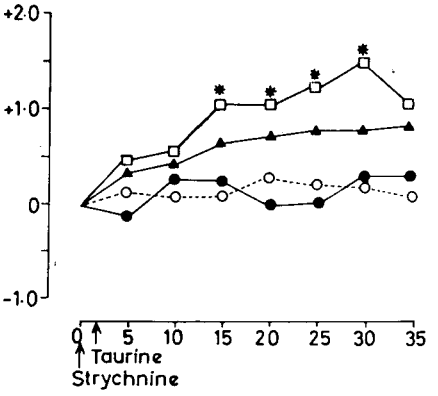

FIG. 3. Time course of the core temperature (ordinate: △ °C) response to bilateral intrahypothalamic combinations of taurine and strychnine. Strychnine (or vehicle) (i.h.) was followed by an immediate injection of taurine (5 μg or vehicle, i.h.). Taurine (5 μg) alone (□), 5 μg strychnine + taurine (▲), 15 μg strychnine + taurine (●), 15 μg strychnine alone (○). Each point represents the mean change in rectal temperature (△ °C) for 5 rats. Standard errors were within the range of 10–15% and have been omitted for clarity. * Denotes statistical difference from saline-injected controls (not shown) ($P < 0·05$) (Mann-Whitney U test). Abscissa: time (min).

$P < 0.05$). The temperature of these animals had returned to within the range of the saline-injected controls by 50 min. A lower dose of hypotaurine (5 μg, i.h.) was without significant effect on body temperature. In contrast, GABA (10 μg, i.h.) produced a hypothermic response in rats (-1.3 ± 0.3 °C at 20 min, $P < 0.05$). Glycine (10 μg, i.h.) was without significant effect on body temperature in rats.

Release of taurine from prelabelled hypothalamic slices

The spontaneous efflux of [^{3}H]taurine was 0·75% of tissue stores min^{-1} compared with an efflux of 5·3% min^{-1} during potassium stimulation (Fig. 4). The peak potassium evoked release in the absence of calcium (<0·1 mM Ca^{2+} 1 mM Mg^{2+}) was significantly reduced to 2·25% min^{-1} ($P < 0.01$).

Taurine uptake into hypothalamus

[^{3}H]Taurine was accumulated into hypothalamic slices by an apparent high-affinity uptake system with calculated K_m value of 8·5 μM and a V_{max} of 29·5 nmol g^{-1} h^{-1}.

DISCUSSION

We have shown that taurine (i.c.v.) produces a hyperthermic response in rats. When injected into the preoptic region of the anterior hypothalamus this amino acid evoked a biphasic dose-related response, with hyperthermia at the lower doses and hypothermia at a higher dose. Biochemically [^{3}H]taurine was released from the hypothalamus by a depolarizing stimulus (50 mM K^+) in a calcium-dependent fashion, and accumulated by an apparent high-affinity uptake system.

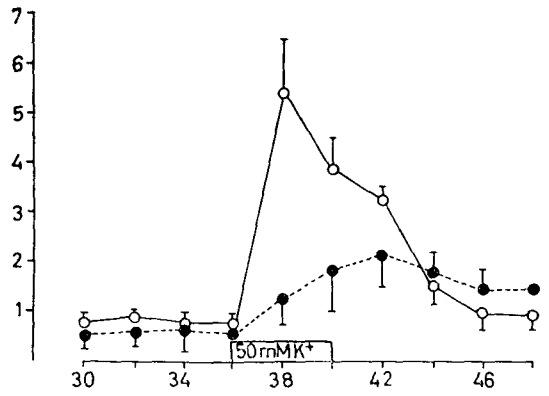

FIG. 4. Release of radioactivity from superfused slices of rat anterior hypothalamus prelabelled with [^{3}H]-taurine in vitro. Potassium chloride (50 mM K^+) evoked a significant calcium-dependent increase in release of radioactivity (○). Dotted lines show experiments performed in the absence of calcium (1 mM Mg^{2+}, <0·1 mM Ca^{2+}) (●). The results are shown as a fractional rate constant derived from the recovered radioactivity and expressed as a fraction of total radioactivity in the tissue as that instant. Each point is the mean of 4 determinations: vertical bars denote standard errors of the mean. Abscissa: time (min).

In other body temperature studies, only a hypothermic response to taurine has been described. These experiments, however, employed massive doses of taurine administered either peripherally to mice (1–6 g kg^{-1}) (Hruska et al 1973, 1976) or intracerebroventricularly to rats (10–40 μmol, i.e. 1·2–5 mg) (Sgaragli & Pavan 1972).

From our results it would seem that a more specific response to centrally-administered taurine is hyperthermia. The hypothermia seen after i.p. injection or higher doses of intrahypothalamic taurine may result from diffusion to other c.n.s. sites involved in temperature regulation. For instance the ventrobasal thalamus is a region associated with behavioural thermoregulatory responses (Hayward 1975). Indeed, such biphasic responses are not completely without precedent and have been reported for several putative transmitters, including noradrenaline and acetylcholine, widely accepted as having a physiological role in temperature regulation (for review, see Cox & Lomax 1977). Perhaps in addition a certain cautionary note should be added in view of the observations of Poole & Stephenson (1977) who noted regular recordings of increased body temperature when measurements were made using a rectal probe. However, in our study the drug-induced hyperthermia usually lasted only 20 min and then returned to within control values, whereas Poole & Stephenson (1977) noted rises for much longer periods. Also, biphasic responses were often seen as well as comparisons being made with the appropriate saline-injected controls. One difficulty in postulating a taurine receptor-mediated response is that there are no specific taurine receptor antagonists (De Feudis 1975). However, the glycine antagonist strychnine has been reported to block the action of taurine on single neurons (Bonaventure et al 1974, Krnjević & Puil 1976). In our study relatively high doses of strychnine (> 5 μg) abolished taurine-induced hypothermia.

We were also able to demonstrate a hyperthermic response to intrahypothalamic hypotaurine. By comparison with taurine itself, hypotaurine was not so potent but exerted a longer duration of action. Although hypotaurine is detectable in brain (Perry & Hansen 1973) and exerts a similar albeit less potent

(5–10 times less potent) inhibitory action as taurine on the isolated immature rat spinal cord (Evans & Watkins personal communication), it is conceivable that the physiological responses of hypotaurine are in fact mediated by its oxidation to taurine itself. Such a conversion may explain its weaker effect but longer duration of action. Of the other inhibitory amino acids studied, intrahypothalamic GABA resulted in hypothermia while glycine had no effect. However, in another report, high doses of both these amino acids administered intracisternally produced a hypothermic response (Sgaragli & Pavan 1972).

These observations so far can only provide circumstantial evidence that taurine is a hypothalamic neurotransmitter involved in thermoregulation. Therefore, in order to seek more direct biochemical evidence that taurine may at least have a hypothalamic neurotransmitter role we have studied the release and uptake of [^{3}H]taurine from hypothalamic slices in vitro. Calcium-dependent depolarization evoked transmitter release is widely accepted as characterizing release from nerve terminals (Rubin 1970). Therefore our observation that potassium stimulated the release of [^{3}H]taurine in a calcium-dependent fashion supports the suggestion that taurine is a possible transmitter candidate in the hypothalamus. Similarly, release of taurine has been demonstrated from cortical preparations in a calcium-dependent fashion both in vitro (Kaczmarek & Davison 1972) and in vivo (Kaczmarek & Adey 1974). Taurine was accumulated into hypothalamic slices by a high-affinity uptake mechanism (K_m = 8·5 μM), a value well within the range for transmitter specific high-affinity uptake (<50 μM) (Balcar & Johnston 1973). Such observations support other reported values for taurine uptake into cortical slices (Kaczmarek & Davison 1972) and rat brain synaptosomes (Sieghart & Karobath 1974; Hruska et al 1978). Thus, taking these biochemical observations into consideration in conjunction with taurine's effect on core temperature, it is suggested that taurine may be a transmitter in the thermoregulatory pathways in the rat hypothalamus.

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