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# Studies on thyrotropin-releasing hormone-induced micturition in cats

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In unanaesthetized cats micturition produced by thyrotropin-releasing hormone (TRH) was investigated after its injection into the cerebral ventricles through chronically implanted cannulae. TRH in doses from 0·1 to 1·0 mg evoked dose-dependent micturition. In cats treated with intracerebroventricular (i.c.v.) reserpine and 6-hydroxydopamine, but not with i.c.v. 5,6-dihydroxytryptamine and hemicholinium, the micturition caused by i.c.v. TRH was abolished. Chlorpromazine and antazoline injected into the cerebral ventricles prevented the micturition induced by i.c.v. TRH. On the other hand, mecamylamine, yohimbine, propranolol, atropine and methysergide injected i.c.v. had virtually no effect or partially antagonized the micturition evoked by TRH similarly injected. It is apparent therefore that centrally induced TRH micturition could be related to central catecholaminergic mechanisms.

Micturition has previously been described following injections of thyrotropin-releasing hormone (TRH) into the cerebral ventricles of cats (Metcalf 1974; Krstić et al 1983; Tomić-Beleslin et al 1985). However, little attention has been paid to the underlying neuropharmacological mechanisms. The present study was undertaken, therefore, to investigate the underlying neuropharmacological mechanisms of micturition produced by TRH injected into the cerebral ventricles of unanaesthetized cats.

*Materials and methods*
Cats of either sex, 2–4 kg, were anaesthetized using sodium pentobarbitone (35–40 mg kg⁻¹, intraperitoneally). Following aseptic precautions, a hole was drilled 7–8 mm from the stereotaxic zero line and 4–5

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* Correspondence.

mm from the mid-line. A Collison cannula was then screwed into the calvarium, so the tip of the cannula rested in the left lateral ventricle (Feldberg & Sherwood 1953). The lower end of the cannula shaft was made of polyethylene tubing with a side opening 1 mm from its closed tip and positioned with the lumen facing the foramen of Monro. Postoperatively, penicillin was administered intramuscularly. An interval of five days elapsed after surgery before an experiment was begun. Post mortem dye studies indicated that the injected material passed from the lateral ventricle into the third and fourth ventricle.

Experiments for i.c.v. injections of TRH and pharmacological antagonists were carried out on cats of either sex, between 2–4 kg (n = 12). Successive injections of either TRH or pharmacological antagonists were separated by an interval of 72 h or longer. The specific regimen for the i.c.v. injections of TRH or pharmacological antagonists was randomized so that each animal was included in each of the experimental conditions. Each cat was used in 3–5 experiments.

In the separate series of experiments to study the effect of inhibitors of acetylcholine, catecholamines and 5-hydroxytryptamine synthesis on micturition evoked by i.c.v. TRH, cats of either sex, 2–4 kg (n = 24) were used. The effect of i.c.v. reserpine on the micturition response to i.c.v. TRH was evaluated over 24 h after a single injection of 1·0 mg, while the effect of i.c.v. 6-hydroxydopamine on the same response to i.c.v. TRH was evaluated over 10–14 days after two consecutive days of treatment with 6-hydroxydopamine in daily doses of 2·0 mg. Hemicholinium, 0·05 mg, was injected

i.c.v. twice daily for 5 days, whereas the effect of i.c.v. 5,6-dihydroxytryptamine on micturition evoked by i.c.v. TRH was evaluated over 10–14 days after two consecutive days of treatment in daily doses of 0·2 mg of 5,6-dihydroxytryptamine. Each animal was used only once.

On the test day, before any behavioural activity and micturition was recorded, the cat was acclimatized to the environment in a wire-mesh cage measuring 110 × 130 × 150 cm for at least 1 h before i.c.v. drug injections. The behaviour of animals was under direct observation continuously throughout the experiments for 4 h and intermittently for 24 h.

Each of the substances injected i.c.v. was dissolved in sterile, pyrogen-free 0·9% sodium chloride solution. The solution was injected manually from a 1·0 mL syringe in a volume of 0·1 or 0·2 mL over a period of 15–20 s and washed in with 0·1 mL of saline. The i.c.v. volume injected never exceeded 0·3 mL, although the critical volume is about 0·7 mL, but it still did not produce any visible behavioural changes in the cat (Krstić & Beleslin 1982).

The compounds used were: synthetic thyrotropin-releasing hormone (Relisorn T, Serono, Rome), atropine sulphate, mecamylamine hydrochloride, yohimbine chloride, propranolol chloride, chlorpromazine chloride, methysergide bimaleate, antazoline chlorhydrate, reserpine (Serpasil, Ciba-Geigy), 6-hydroxydopamine bromide, 5,6-dihydroxytryptamine creatine sulphate and hemicholinium-3. 6-Hydroxydopamine and 5,6-dihydroxytryptamine were dissolved in 0·9% saline containing 0·1 mg mL$^{-1}$ ascorbic acid. All drug doses refer to the salts except those of thyrotropin-releasing hormone which refer to the tripeptide, while that of reserpine refers to the drug.

Dose-response curves were calculated using linear regression according to the method of least squares. A coefficient of correlation (r) of linear regression was used to determine the existence of dose-reponse. Student's *t*-test was used to determine the significance of the difference between controls and various experimental groups. The results were considered statistically significant when $P < 0.05$.

*Results*
As shown in Fig. 1A TRH (0·1–1·0 mg) injected into the cerebral ventricles of the cat induced dose-dependent micturition (r = 0·96; $P < 0.05$). However, the percentage of micturition, even with the largest doses of TRH (1·0 mg), never reached 100% (Fig. 1A). The average latencies ranged from 30 to 100 s.

In addition to micturition, animals treated with i.c.v. TRH exhibited the usual autonomic, motor and behavioural responses.

When TRH was injected i.c.v. repeatedly at intervals of 72 h the most consistent response was obtained with doses of 0·4 mg. Therefore this dose was used as a control value for the neuropharmacological analysis of
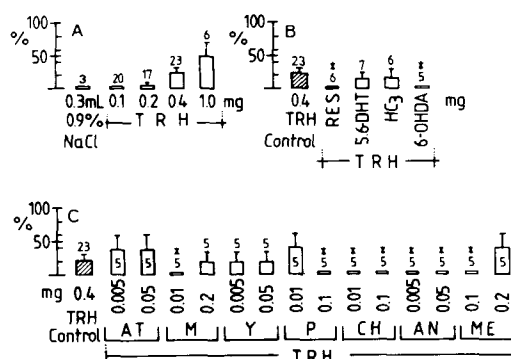


FIG. 1. A, micturition produced by TRH injected into the cerebral ventricles of the cat. B, the effect of reserpine (RES), 5,6-dihydroxytryptamine (5,6-DHT), hemicholinium (HC-3) and 6-hydroxydopamine (6-OHDA) injected intracerebroventricularly on micturition induced by TRH similarly injected. C, the effect of atropine (AT), mecamylamine (M), yohimbine (Y), propranolol (P), chlorpromazine (CH), antazoline (AN), and methysergide (ME) injected into the cerebral ventricles on micturition evoked by TRH injected intracerebroventricularly. Ordinates: percentage of animals showing micturition. On abscissa in B and C the first column (hatched) represents the control value for micturition. In A and C each column represents mean ± s.e. of 5–23 injections, whereas in B each column, except the control, represents mean ± s.e. of 5–7 cats. The number of injections and cats at each dose denoted at columns in A, B and C. Differences from control value are significant at *$P < 0.05$.

micturition evoked by i.c.v. TRH. In cats treated with both i.c.v. reserpine and 6-hydroxydopamine the micturition caused by i.c.v. TRH was abolished (Fig. 1B). On the other hand, in cats treated either with i.c.v. 5,6-dihydroxytryptamine or hemicholinium the micturition produced by i.c.v. TRH was virtually unchanged (Fig. 1B).

The antimuscarinic drug, atropine (0·005–0·05 mg), the ganglion blocking drug, mecamylamine (0·01–0·2 mg), the α-adrenoceptor blocking drug, yohimbine (0·005–0·05 mg), the β-adrenoceptor blocking drug, propranolol (0·01–0·1 mg), the dopamine antagonist, chlorpromazine (0·01–0·1 mg), the antihistamine, antazoline (0·005–0·05 mg) and the 5-hydroxytryptamine antagonist, methysergide (0·1–0·2 mg) were used to prevent the micturition induced by TRH (0·4 mg). The antagonists were injected i.c.v. 15–20 min before TRH was similarly injected. As shown in Fig. 1C the micturition caused by TRH was prevented by chlorpromazine and antazoline, whereas mecamylamine and methysergide in small doses, as well as propranolol in large doses, abolished the micturition evoked by TRH. On the other hand, atropine and yohimbine had virtually no effect on TRH-induced micturition (Fig. 1C).

Injections of 0·2 or 0·3 mL of 0·9% saline into the cerebral ventricles of unanaesthetized cats (n = 3) did not produce any visible behavioural, autonomic or motor phenomena. In addition, two repeated i.c.v.

injections of 0·9% saline (n = 4) in volumes of 0·2 or 0·3 mL at intervals of 15–20 min did not evoke any visible behavioural, autonomic or motor changes.

*Discussion*

TRH, in doses from 0·1 to 1·0 mg, injected into the cerebral ventricles of unanaesthetized cats is reported to produce micturition (Metcalf 1974; Krstić et al 1983; Tomić-Beleslin et al 1985). The results of the present experiments further show that the micturition evoked by i.c.v. TRH is dose-dependent. Apart from micturition, in the present experiments as well as in the experiments previously reported, TRH injected into the cerebral ventricles or microinjected into the reticular substance of the brainstem of the cat produced similar responses to those reported by Metcalf (1974); Metcalf & Myers (1976); Myers et al (1977); Krstić et al (1983); Tomić-Beleslin et al (1985). It appears therefore that the micturition induced by TRH injected into the cerebral ventricles of the cat is associated with other autonomic phenomena.

In the experiments reported here i.c.v. reserpine and 6-hydroxydopamine, but not i.c.v. 5,6-dihydroxytryptamine and hemicholinium prevented the micturition evoked by TRH injected into the cerebral ventricles. These findings are in line with those in which it was reported that central catecholaminergic mechanisms appear to be involved in the effects of TRH in the brain (Keller et al 1974; Heal & Green 1979; Rastogi et al 1980). In this context, i.c.v. reserpine, 6-hydroxydopamine, 5,6-dihydroxytryptamine and hemicholinium blocked the micturition evoked by the nicotinic ganglionic agonists, nicotine and dimethylphenylpiperazinium injected into the cerebral ventricles of unanaesthetized cats (Krstić et al 1985; Beleslin & Krstić 1985). In the cat, areas of the micturition reflex centre have been described in the brainstem and diencephalon (Tang & Ruch 1956; Satoh et al 1978). Since the brainstem contains catecholamine-containing neurons (Dahlström & Fuxe 1964) it appears that at least one mechanism contributing towards TRH-induced micturition is suggested.

It is presently unclear how TRH activates the central catecholaminergic system to produce micturition. There are at least two possibilities. The finding that chlorpromazine, but not yohimbine and propranolol, in smaller, as well as in larger doses, prevented the TRH-induced micturition indicates that TRH could have acted via dopaminergic receptors. However, this antagonism does not seem to be specific since the antihistamine, antazoline antagonized also the micturition evoked by TRH. Furthermore, specific TRH binding sites have been found in the brain (Burt & Snyder 1975; Ogawa et al 1982; Mantyh & Hunt 1985) and therefore the tripeptide could activate the catecholaminergic system through these sites. It follows then that TRH could play an important role in the regulation of micturition via an action on the central catecholaminergic system. Finally, the results of the present experiments favour a transmitter or a modulator role of the tripeptide, although the physiological importance of TRH in the regulation of micturition is not yet understood.

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