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# The hypothermic action of carbachol in the rat brain periaqueductal grey area may involve neurotensin

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**1** Neurotensin (NT) and carbachol both caused hypothermia when injected into the periaqueductal grey area (PAG) of rat brain.

**2** Atropine prevented carbachol- but not NT-induced hypothermia.

**3** NT-induced hypothermia was unaffected by various neurotransmitter agonists and antagonists in the PAG.

**4** Both NT antibodies and thyrotrophin releasing hormone prevented carbachol hypothermia.

**5** It is concluded that the hypothermic action of carbachol in the PAG is mediated via endogenous NT.

## Introduction

Neurotensin (NT) is an endogenous tridecapeptide shown to be widely but unevenly distributed throughout the mammalian central nervous system (Uhl & Snyder, 1976; Uhl *et al.*, 1977). The peptide may be a neurotransmitter or neuromodulator (Brown & Miller, 1982), and following CNS administration, NT produces a variety of behavioural and other effects (St Pierre *et al.*, 1984). One of its more potent actions is the induction of hypothermia after intracisternal or intraventricular injection (Bissette *et al.*, 1976; Nemeroff *et al.*, 1977; Mason *et al.*, 1980). The hypothermic effect can be reproduced by direct application of NT to both the hypothalamus and the periaqueductal grey region (PAG) (Martin *et al.*, 1980). NT is concentrated within the midbrain central grey (Uhl *et al.*, 1979), where there are numerous specific NT binding sites (Uhl & Snyder, 1977). Interactions between NT and established brain neurotransmitters, especially dopamine, have been shown (Haubrich *et al.*, 1982) but nothing is known about the mechanisms underlying the body temperature effects of the peptide. In the present study, the effects of some pharmacological agents and specific NT antibodies on the hypothermia induced by NT in the PAG have been investigated in order to understand more fully the mode of action of this potent neuropeptide.

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## Methods

### *Injections in the periaqueductal grey region*

Adult male Sprague-Dawley rats (180–200 g) were anaesthetized with 50 mg $kg^{-1}$ methohexitone sodium (Brietal, Lilly) and placed in a stereotaxic frame. By means of co-ordinates derived from the atlas of König & Klippel (1963), a guide cannula made from 23-gauge stainless steel tubing was implanted in the skull above the PAG, and fixed with dental cement to screws set in the skull. The guide was positioned so that an injection cannula made from 31-gauge stainless steel tube insert into the guide extended approximately 1 mm beyond the guide into the PAG at the co-ordination A 0.6, H −0.5, L 0.45. Rats were housed in individual cages under standard conditions with free access to food and water. A 7-day post-operative recovery period was allowed before experiments were started. Microinjections of compounds dissolved in sterile 0.9% saline (in a total volume of 1 μl over 5 s) into the PAG were made not more frequently than once every 48 h. Of the drugs injected (see Table 1), methysergide (Sandoz) is unstable when dissolved in saline, so was used immediately after dissolving in sterile saline.

### *Temperature recording*

Experiments were performed at an ambient temperature of 21°C. Temperature measurements were

made by inserting a thermistor probe into the large intestine 6 cm from the anus, and recording the temperature on an electric thermometer. Recordings were taken at 10 min intervals both before and after injections under light physical restraint. This restraint was by gentle placing of the hand over the rat to prevent undue movement while body temperature was taken. Following completion of the experiments, 0.5 μl of diluted Indian ink was injected into the PAG; the rats subsequently received intracardiac infusions first of heparinized saline, and then of 10% neutral buffered formalin. Injection sites were confirmed histologically. Statistical analysis of results was as previously described (Widdowson *et al.*, 1983) and by analysis of variance. Some rats were used for more than one experiment and were permitted a period of at least 4 days between injections. Previous exposure to either carbachol or neurotensin did not alter subsequent responses.

*Materials*

Peptides, synthesized by Bachem Inc. U.S.A., were obtained from Universal Biologicals Ltd, London. Specific antiserum to NT, kindly donated by Dr G.W. Bennett, University of Nottingham, was raised in sheep:- this antiserum has been used extensively for NT radioimmunoassay and shows no cross-reactivity with a large number of hypothalamic peptides and anterior pituitary hormones including luteinizing hormone-releasing hormone, thyrotrophin-releasing hormone (TRH), [Met$^5$]enkephalin, vasoactive intestinal polypeptide, somatostatin, rat thyrotrophin, prolactin and luteinizing hormone, and a 70% cross-reactivity with the [1–8]NT fragment (Sheppard & Shennan, 1983). Drugs used were obtained from the sources listed in Table 1.

## Results

*Neurotensin-induced hypothermia*

NT (0.5–5.0 μg) injected into the PAG of conscious rats caused a dose-related decrease in mean body temperature (Figure 1). The maximum hypothermia was recorded 60–70 min after injection, and was followed by a progressive return to the normal mean

**Table 1** Effect of drugs injected in rat brain periaqueductal grey region (PAG) on body temperature: interactions with saline and neurotensin

| | | *Rectal temperature* (°C) | | |
|---|---|---|---|---|
| | | *Saline in PAG* | *Neurotensin in PAG* (1 μg) | |
| Drug | Dose (μg) | 40 min | 40 min | 100 min |
| – (Saline only) | – | 37.3 ± 0.2 | 35.6 ± 0.1 | 36.5 ± 0.2 |
| 5-Methoxy-*N*,*N*-dimethyltryptamine (Sigma) | 1 | 37.3 ± 0.2 | 35.4 ± 0.1 | 36.9 ± 0.15 |
| Cyproheptidine (Merck) | 2 | 37.1 ± 0.1 | 35.2 ± 0.05 | 36.3 ± 0.05 |
| Methysergide bimaleate (Sandoz) | 2 | 37.3 ± 0.1 | 35.1 ± 0.1 | 36.9 ± 0.1 |
| 5-Hydroxytryptamine creatine sulphate (Sigma) | 1 | 37.2 ± 0.1 | 35.7 ± 0.2 | 36.7 ± 0.2 |
| Glutamate (Sigma) | 2 | 37.1 ± 0.3 | 35.2 ± 0.2 | 36.8 ± 0.1 |
| Apomorphine HCL (Sigma) | 2 | 37.0 ± 0.2 | 35.6 ± 0.1 | 36.9 ± 0.1 |
| (+)-Amphetamine sulphate (Smith, Kline & French) | 5 | 37.2 ± 0.2 | 35.7 ± 0.2 | 36.9 ± 0.15 |
| Haloperidol (Searle) | 5 | 37.2 ± 0.2 | 35.0 ± 0.25 | 36.4 ± 0.15 |
| Muscimol (BDH) | 0.2 | 36.6 ± 0.2 | 35.6 ± 0.3 | 36.6 ± 0.15 |
| (±)-Baclofen (Ciba-Geigy) | 1 | 37.2 ± 0.1 | 35.8 ± 0.15 | 37.2 ± 0.15 |
| Picrotoxin (Sigma) | 0.1 | 37.0 ± 0.1 | 35.7 ± 0.15 | 36.7 ± 0.1 |
| Isoguvacine HCL (BDH) | 1 | 37.2 ± 0.2 | 35.8 ± 0.1 | 37.2 ± 0.15 |
| Isoprenaline sulphate (Macarthy's) | 5 | 37.3 ± 0.1 | 35.2 ± 0.2 | 36.5 ± 0.25 |
| (−)-Noradrenaline bitartrate (Koch-Light) | 2.5 | 37.2 ± 0.1 | 35.6 ± 0.1 | 35.9 ± 0.05 |
| Phentolamine mesylate (Ciba-Geigy) | 2 | 37.1 ± 0.1 | 35.5 ± 0.05 | 36.9 ± 0.1 |
| Atropine methylnitrate (BDH) | 5 | 37.1 ± 0.2 | 35.2 ± 0.3 | 36.2 ± 0.2 |
| Carbachol chloride (BDH) | 5 | 35.2 ± 0.3* | 33.9 ± 0.3* | 34.8 ± 0.5* |
| Indomethacin (Merck) | 2 | 36.9 ± 0.2 | 35.7 ± 0.1 | 36.7 ± 0.1 |
| Naltrexone HCL (Endolabs) | 2 | 36.6 ± 0.3 | 35.2 ± 0.2 | 36.3 ± 0.25 |

Each value is the mean ± s.e.mean; $n = 6–8$. Source of drug in parentheses. *$P < 0.01$ vs. saline only at appropriate time, by analysis of variance.

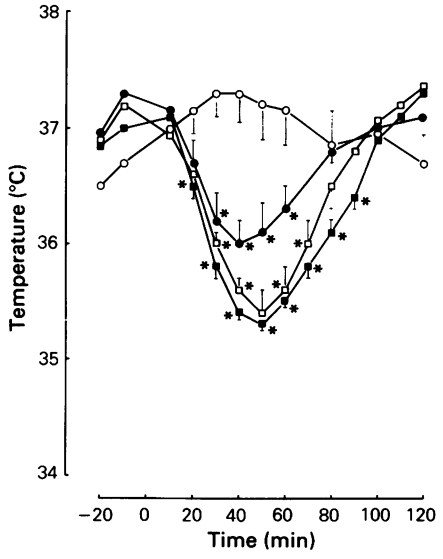


**Figure 1** Body temperature effects of saline (1 μl, ○) and neurotensin (0.5 μg, ●; 1 μg □; 5 μg ■) injected at time zero into the periaqueductal grey area of rat brain. Each result is the mean value obtained using a group of 6 rats; vertical lines show s.e.mean. The values obtained for the saline- (control) and neurotensin-treated animals were analysed using the Mann-Whitney U test. *Indicates a significant difference ($P < 0.01$) between saline control and peptide-treated animals.

body temperature, which was reached 100 min after injection.

*Effects of pharmacological agents*

Drugs that modify brain γ-aminobutyric acid (GABA), 5-hydroxytryptamine (5-HT), glutamate, dopamine, noradrenaline and opioid function were injected into the PAG of rat brain, and their effects on body temperature and on NT-induced hypothermia were measured. The drugs employed and the doses given are listed in Table 1, together with the temperature responses recorded. None of these compounds alone had any significant effect on body temperature ($P > 0.05$), or, when injected simultaneously with NT, modified in any way the NT-induced hypothermia.

Two compounds which affect brain cholinergic function, namely atropine and carbachol, were injected into the PAG of conscious rats. Atropine had no effect on body temperature (Table 1), whereas carbachol (0.5–5 μg) in the PAG produced a dose-related fall in mean body temperature (Figure 2). The time course of the carbachol-induced hypothermia was very similar to that of NT, with a maximum effect 50–60 min after injection. Simultaneous injection of atropine (5 μg) and NT (1 μg) in the PAG had no effect on NT-induced hypothermia (Figure 3). However,

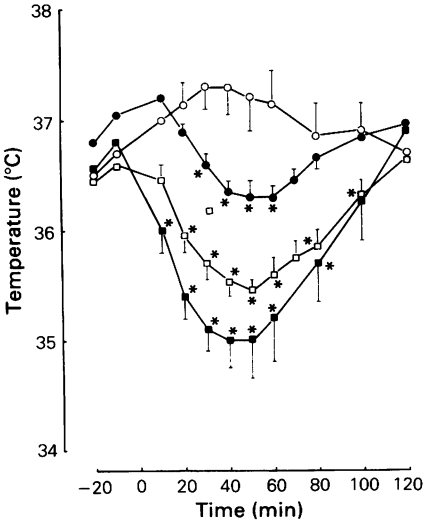


**Figure 2** Body temperature effects of saline (1 μl, ○) and carbachol (1 μg ●, 2.5 μg □, 5 μg ■) injected at time zero into the periaqueductal grey area of rat brain. Each result is the mean value obtained using a group of rats; vertical lines show s.e.mean. *$P < 0.01$; see Figure 1 legend.

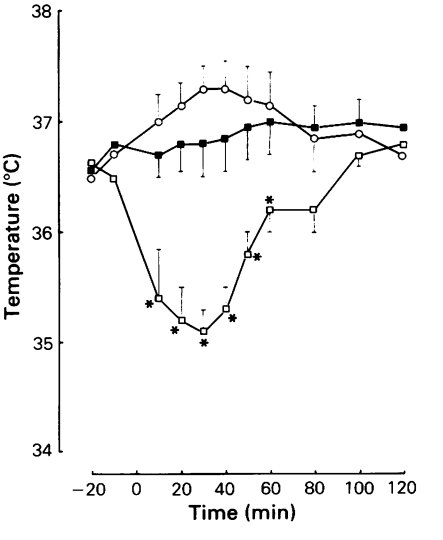


**Figure 3** Body temperature effects of saline (1 μl, ○), carbachol 5 μg plus atropine 5 μg (■) and neurotensin 1 μg plus atropine 5 μg (□) injected at time zero into the periaqueductal grey area of rat brain. Each result is the mean value obtained using a group of rats; vertical lines show s.e.mean. *$P < 0.01$; see Figure 1 legend.

atropine completely prevented the hypothermia produced by carbachol.

The observed similarities between the hypothermia produced by NT in the PAG and that caused by carbachol (potency, time course) prompted examination of the role of endogenous NT in the carbachol-induced fall in temperature. In the absence of an NT receptor antagonist, specific NT antibodies (Sheppard & Shennan, 1983) were used. Rats were pretreated for 5 days with injections of NT antibodies in the PAG (1 μl, 1:10 dilution in 0.9% sterile saline). On day 6, NT (1 μg) or carbachol (5 μg) was injected into the PAG. The antibody pretreatment attenuated the NT-induced hypothermia and entirely prevented the fall in temperature normally produced by carbachol (Figure 4). The normal carbachol-induced hypothermia was replaced by a slowly-developing rise in body temperature.

Many of the central actions of NT are prevented by TRH. For example, TRH readily prevents NT-induced hypothermia (Griffiths *et al.*, 1983). The effect of TRH on carbachol-induced hypothermia was investigated. A low dose of TRH (0.5 μg) in the PAG 10 min before carbachol had little effect on the hypothermia but the larger doses of TRH tested (1 and 5 μg) partly and entirely prevented the hypothermia, respectively (Figure 5). Verification of injection sites took place at the end of experiments: the success rate for cannulating the PAG was 98% and only rats with confirmed cannulation (by subsequent dye injections) were used. The 2% unsuccessfully-cannulated showed no response to neurotensin or carbachol.

## Discussion

Significant amounts of NT are present in the PAG (Emson *et al.*, 1982) and, when applied directly to this brain area, the peptide causes hypothermia, inhibits the response of rats to a nociceptive stimulus and reduces hind-limb muscle tone (Griffiths *et al.*, 1981; 1983; Widdowson *et al.*, 1983). The present findings confirm that local injection of NT into the PAG causes a pronounced hypothermia (Martin *et al.*, 1980), the mechanism of which is unknown (Brown & Miller, 1982). In the present study, no direct evidence was found to implicate any one of several brain neurotransmitters in the hypothermic action of NT, since the hypothermia was not altered by local application to the PAG of compounds modifying 5-HT, dopamine, GABA, noradrenaline, ACh and opioid function, though perhaps not of all the various receptor types

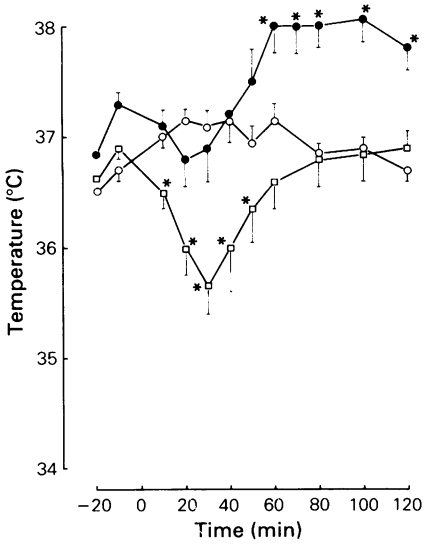


**Figure 4** Body temperature effects of saline (1 μl, ○), neurotensin (1 μg, □) and carbachol (5 μg, ●) injected at time zero into the periaqueductal grey (PAG) area of brain in rats pretreated for 5 days with antibodies to neurotensin into the PAG. Each result is the mean value obtained using a group of rats; vertical lines show s.e.mean. *$P < 0.01$; see Figure 1 legend.

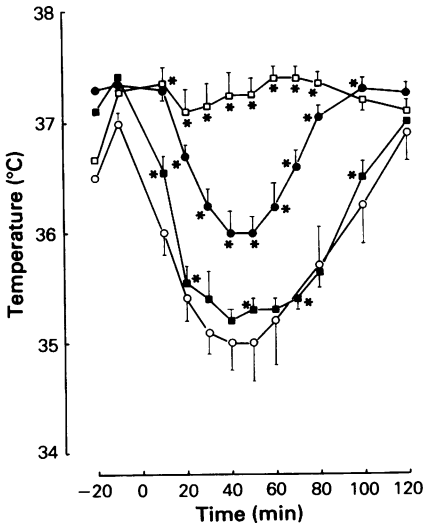


**Figure 5** Body temperature effects of carbachol (5 μg, ○) and carbachol plus thyrotrophin-releasing hormone (TRH) (0.5 μg, ■; 1 μg, ●; 5 μg, □) injected at times −10 min (TRH) and zero (carbachol) into the periacqueductal grey area of rat brain. Each result is the mean value obtained using a group of rats; vertical lines show s.e.mean. *Indicates a significant difference ($P < 0.01$) between carbachol and carbachol plus TRH results.

present in brain. So, it is likely that NT acts upon NT receptors situated on neurones that project from the PAG to other sites in the brain to produce the hypothermia observed.

Carbachol in the PAG appeared to activate atropine-sensitive muscarinic receptors to cause hypothermia, with a similar time-course to that of NT, suggesting NT may be involved in the cholinergic hypothermia. However, atropine did not influence NT-induced hypothermia, excluding the possibility that NT might act via a central cholinergic mechanism antagonized by atropine. To prove an acetylcholine (ACh)-NT link in the PAG responsible for carbachol hypothermia would require a specific antagonist for the NT receptor. Since there are no such compounds yet available, specific antibodies to NT were employed as a means of eliminating endogenous NT, since they would bind and inactivate the neuropeptide (Shennan & Sheppard, 1983). Antibody pretreatment reduced, but did not entirely prevent the hypothermic action of exogenous NT. More importantly, the NT antibodies prevented the carbachol-induced hypothermia, suggesting that the carbachol response may be dependent upon endogenous NT in the PAG and providing evidence of an ACh-NT link in this brain area. In the NT antibody-pretreated rats, carbachol failed to produce hypothermia. Instead, a slow-developing and sustained hyperthermia was observed, which might be attributed to facilitation of a hyperthermic mechanism because of the removal of endogenous NT by the antibodies. A small, but not significant, rise in body temperature was seen in the saline controls, which might well be due to the experimental procedures.

Although there are no specific NT receptor antagonists, TRH often functions as an inhibitor of NT-induced responses in the CNS (Griffiths *et al.*, 1981; Widdowson *et al.*, 1983): TRH prevented the NT-induced hypothermia in the PAG (Widdowson *et al.*, 1983). In the present study, TRH prevented the carbachol-induced hypothermia in a similar manner to that already observed for NT. In view of the mutual antagonism between TRH and NT in several centrally-mediated responses, the effect of TRH on carbachol-hypothermia provides another indirect indication of the connection between NT and ACh in the PAG.

Although the PAG is a brain area intimately concerned in pain perception, another role seems to be thermoregulation. For both functions, the PAG is well-supplied with endogenous neuropeptides, for in addition to NT, this brain area also contains TRH and β-endorphin (Finley *et al.*, 1981; Jackson, 1982). Although TRH in the PAG may not play a direct role in thermoregulation, it is capable of preventing or modulating the actions of NT (Griffiths *et al.*, 1981; Widdowson *et al.*, 1983). Thus, endogenous NT neurones in the PAG which function in thermoregulation may be subjected to a dual influence by both TRH and cholinergic neurones. At present, it is known that the PAG contains muscarinic receptors as well as significant amounts of endogenous ACh (Rotter *et al.*, 1979). What is less certain is the identity of the neuronal pathways from the PAG that are involved in thermoregulation. Ascending and descending efferent projections have been identified in the monkey and cat but little such detailed information exists for the rat PAG (Mantyh, 1982; Bragin *et al.*, 1984). Hence, it is difficult to define which PAG projections in the rat involve NT and ACh. In view of the uncertainty about the exact mechanism of NT-induced hypothermia, this may be a profitable area for future study.

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