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# ANALYSIS OF THE EFFECTS ON BODY TEMPERATURE OF INTRACEREBROVENTRICULAR INJECTION IN ANAESTHETIZED DOGS OF GAMMA-AMINOBUTYRIC ACID

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1 The cerebral ventricles of dogs under intravenous pentobarbitone sodium anaesthesia, were perfused with artificial cerebro-spinal fluid (CSF) at a rate of 0.4-0.5 ml/min from the ventricular to the aqueductal cannulae. The effluent was collected from the aqueductal cannula in 20 min samples. The animals' temperatures were recorded from the rectum.

2 γ-Aminobutyric acid (GABA) 0.1-5 mg when injected into the ventricles produced variable temperature effects. Doses of 0.1 and 0.5 mg always produced hyperthermia and 1 and 5 mg doses sometimes produced hyperthermia and sometimes hypothermia.

3 Intraventricular perfusion with 2-bromolysergic acid diethylamide (BOL) and hyoscine did not block hyperthermia. Tests on the rat isolated stomach strip or the guinea-pig isolated superfused ileum for the possible release, respectively, of 5-hydroxytryptamine or acetylcholine by GABA were negative.

4 When tested for the presence of prostaglandin E(PGE)-like substances on the isolated rat stomach strip, both the control effluent and the GABA effluent showed activity, the latter being much more potent. There was a temporal correlation between this effect and hyperthermia. Intraventricularly administered sodium salicylate converted the GABA-induced hyperthermia to hypothermia and blocked the release of PGE-like substances.

5 Hypothermia induced by GABA alone or in the presence of sodium salicylate was associated with the release of noradrenaline into the effluent.

6 Intraventricular administration of GABA in reserpinized dogs produced hyperthermia and not hypothermia. Similar results were obtained with phentolamine perfusion in normal dogs.

7 Perfusion with calcium-free solution blocked both the noradrenaline-releasing and hypothermic actions of GABA.

8 It is concluded that hyperthermia associated with intraventricular injections of GABA is due to the release of PGE-like substance and hypothermia is due to the release of noradrenaline.

## Introduction

The hypothalamus contains noradrenaline and adrenaline (Vogt, 1954), 5-hydroxytryptamine (Garattini & Valzelli, 1965), acetylcholine (MacIntosh, 1941), γ-aminobutyric acid (GABA) (Berl & Waelsh, 1958) and prostaglandin $E_1$ ($PGE_1$) (Holmes & Horton, 1968). The effects on body temperature of intraventricularly or intrahypothalamically injected noradrenaline and adrenaline, 5-hydroxytryptamine, acetylcholine and $PGE_1$ have been extensively investigated in several species of animals (Feldberg & Myers, 1963, 1964a, 1964b, 1965; Feldberg, Hellon & Lotti, 1967; Myers, 1967; Cooper, Granston & Honour, 1965; Bligh, 1966; Findlay & Robertshaw, 1967; Anderson, Jobin & Olsson, 1966; Brittain & Handley, 1967; Myers & Yaksh, 1969; Feldberg & Saxena, 1971a, 1971b; Milton & Wendlandt, 1971).

Except for a recent brief report (Komaromi, Moore & Sinanan, 1969) which described the hyperthermic effect of GABA administered intraventricularly in the neonatal rabbit, there is no information on the effects of this compound on body temperature in the available literature.

In the present study, the effects of intraventricularly administered GABA were examined on the

body temperature of unanaesthetized and anaesthetized dogs. An attempt has also been made to elucidate, in anaesthetized dogs, the mechanisms of the temperature effects of GABA.

## Methods

*Perfusion of the cerebral ventricles*

Male and female mongrel dogs (6-10 kg) were anaesthetized with intravenous pentobarbitone sodium (30 mg/kg). The trachea and femoral vein were cannulated and the dog's head was placed in a clamp. The skin, connective tissue and muscles were removed from the top of the skull. A cannula (No. 21 B.D. needle) was placed with its tip into the left lateral ventricle by introducing it to a depth of about 24 mm from the surface of the skull. A reasonably consistent placement of the cannula was indicated by the free flow of cerebrospinal fluid (CSF) and the rhythmic movements of the fluid, synchronous with the respiratory movements. To insert the second cannula, the layers of muscles covering the atlanto-occipital membrane were dissected away and the membrane cut so as to expose the medulla. The lower part of the cerebellum was exposed by nibbling away the margins of the supraoccipital bone at the lower border of foramen magnum. This made it possible to lift the cerebellum gently and insert a fine polythene tube along the floor of the fourth ventricle into the aqueduct. The outer diameter of this tube was 2 mm, which was narrowed to 1 mm at its tip. The tube was inserted for at least 20 mm beyond the margin of the cerebellum and the position of the tip of the cannula was ascertained at the end of each experiment as follows: the perfusion was stopped and 1 ml of 1% methylene blue solution was slowly injected with a syringe through the ventricular cannula. If the cannula was in the right position, the dye flowed out only through the polythene tube. If traces of dye seeped out around the tube, then the tip of the cannula had not reached the aqueduct. A further check was made after killing the dog by dissecting the brain without removing the tube and locating its tip in the aqueduct. A three-way stop cock was attached to the ventricular cannula. Artificial CSF (NaCl, 8.1 g; KCl, 0.25 g; $CaCl_2$, 0.14 g; $MgCl_2$, 0.11 g; $NaHCO_3$, 1.76 g; $NaH_2PO_4$, 0.07 g; glucose, 0.61 g; urea, 0.13 g; and distilled water to make one litre; pH 7.1) as described by Merlis (1940) was perfused through the brain from the left lateral ventricle to the aqueduct by gravity at a rate of 0.4-0.5 ml/minute. The first 60 min effluent was discarded. Subsequently the effluent was collected in 20 min samples. When the effluent was to be assayed for its noradrenaline content, it was collected in 3 ml of 0.4 N perchloric acid.

GABA, acetylcholine and physostigmine, $PGE_1$ and noradrenaline were injected slowly in volumes of 0.5 ml through the side arm of the three-way stop cock. The perfusion was stopped during the period of injection. The effluent collected after the intraventricular injection of GABA will, hereafter, be referred to as GABA effluent.

In some experiments, the ventricles were perfused with drugs and in others, the ventricles were perfused with artificial CSF lacking in calcium. Collection of the effluent samples was begun 10 min after starting perfusion with drugs.

*Record of rectal temperature*

A thermistor probe was inserted 80 mm into the rectum and connected to 'Elima Temperature Recorder' (Hartmann & Braun). The probe was held in position by adhesive tape attached to the protruding end of the probe and gently wrapped round the base of the tail. The temperature was continuously recorded and the figures reproduced in this study are plotted from these records. The room temperature ranged from 28 to 33° C.

*Tests for the presence of 5-hydroxytryptamine and prostaglandin-like substances in the effluent*

Thirty to 40 mm long preparations of stomach or colon were obtained from rats of either sex weighing 150-200 g. The isolated stomach strip as described by Vane (1957) was used for testing the presence of 5-hydroxytryptamine and PGE-like substances in the effluents. Rat colon preparations, set up according to the method of Regoli & Vane (1964), served to exclude the presence of PGF-like substances in the effluents. The preparations were set up in 15 ml baths and bathed in Krebs bicarbonate solution at 37° C, bubbled with a mixture of 5% $CO_2$ and 95% $O_2$. Movements were recorded on smoked kymograph paper with an isotonic lever under 500 mg tension and 15-fold magnification. A 10 min response cycle was used.

*Tests for the presence of acetylcholine in the effluent*

The effluent was tested for the presence of acetylcholine on the superfused terminal ileum obtained from male guinea-pigs weighing 300-450 g. Tyrode solution at 35° C superfused a 2 cm long piece of ileum at a rate of 2 ml/min and contained hexamethonium (20 μg/ml), mepyramine (0.5 μg/ml) and morphine (1 μg/ml). The contractions were recorded on smoked paper with

an isotonic frontal lever under 500 mg tension and were magnified 8-fold. A 3 min response cycle was used.

*Tests for the presence of noradrenaline in the effluent*

The noradrenaline content of the effluent was estimated by the method of Anton & Sayre (1962). Fluorescence was measured in a Turner fluorometer (Model 110). The values presented in this paper are corrected for a 70% recovery of noradrenaline.

*Reserpine pretreatment*

Reserpinized animals had been injected with 0.3 mg/kg of reserpine (Serpasil, Ciba) intramuscularly 72 h before the start of an experiment.

*Unanaesthetized dogs*

Two dogs were lightly anaesthetized with ether and Collison cannulae were permanently implanted into the left lateral ventricles under aseptic surgical procedures by the method of Feldberg & Sherwood (1953). The cannula was secured to the skull by dental acrylic and was closed by a cap fitted with a rubber diaphragm. Injections were made one week after the implantation of the cannula by a needle inserted through the rubber diaphragm. The method of recording the temperature was the same as described above.

*Drugs*

Acetylcholine chloride (E. Merck); 2-bromolysergic acid diethylamide (BOL; Sandoz); γ-aminobutyric acid (GABA; Calbiochem); hexamethonium chloride (Sarabhai Chemicals); histamine dihydrochloride (Calbiochem); 5-hydroxytryptamine creatinine sulphate (Sandoz); hyoscine hydrochloride (E. Merck); (±)-isoprenaline sulphate (Ward, Blenkinsop & Co.); mepyramine maleate (May & Baker); morphine hydrochloride (May & Baker); (−)-noradrenaline (Fluka, AG); pentobarbitone sodium (Rhone-Poulenc); phenoxybenzamine hydrochloride (Smith, Kline & French); phentolamine methanesulphonate (Ciba); (±)-propranolol hydrochloride (I.C.I.); prostaglandin $E_1$ ($PGE_1$; Upjohn); reserpine (Serpasil; Ciba); sodium edetate (B.D.H.); sodium salicylate (Alta Laboratories).

## Results

*Unanaesthetized dogs*

GABA (25 μg) given intraventricularly in 0.5 ml of artificial CSF produced a 1.3°C rise in temperature in one dog and a 1.2°C rise in another. The rise began almost immediately and reached a peak in about 75 min; the temperature remained elevated for up to 90 min after which time it began to fall, returning to the control level by about 135 minutes. The dogs were highly active and excited. There was marked dilatation of the pupils, vocalization, salivation, licking and sniffing, defaecation and urination. Intraventricular injection of 0.5 ml of artificial CSF produced urination.

*Anaesthetized dogs*

The control outflow from the cannula ranged between 5-8 ml/20 minutes. Following GABA injections, the outflow ranged between 3.5-6.3 ml/20 minutes.

With 0.1 mg to 5 mg of GABA injected into the perfusion cannula, there was a mean rise in temperature of 0.25 to 0.66°C within 2-3 min (Table 1); there was no dose-response relationship. The rise was minimal after 1 mg of GABA and in one of the three experiments with this dose, there was a fall in temperature. Other effects sometimes associated with the intraventricular injections of GABA were an increase in the rate and amplitude of respiration, urination, defaecation, salivation and shivering.

*Effect on rectal temperature of intraventricular injection of GABA in the presence of phentolamine and BOL or phentolamine, BOL and hyoscine perfused into the ventricles.* A mixture of 50 μg each of acetylcholine and physostigmine injected intraventricularly produced a 0.3°C fall in temperature which was short-lived (one experiment). In three experiments each, perfusion of the ventricles with phentolamine (20 μg/ml) and BOL (4 μg/ml) or phentolamine, BOL and hyoscine (20 μg/ml), either produced hyperthermia (Fig. 1) or had no effect. GABA, 0.1-5 mg given during such a perfusion produced hyperthermia. The 5 mg dose of GABA given during perfusion with phentolamine and BOL produced a rise in temperature of 1 ± 0.08°C (Fig. 1) and when hyoscine was also present in the perfusion fluid a rise of 0.8 ± 0.4°C. Other effects exhibited by these animals following 5 mg of GABA were salivation, tremors, defaecation and shivering.

*Investigation of the possible release of 5-hydroxytryptamine into the effluent following intraventricular injection of GABA.* 5-Hydroxytryptamine (5-10 ng), 0.25 ml of the control effluent and 0.25 ml of the GABA effluent (5 mg dose), added after an equilibration period of 2 h, contracted the isolated rat stomach strip. The contractions produced by the GABA effluent were

**Table 1** Effect of intraventricular injections of γ-aminobutyric acid (GABA) on the rectal temperatures of anaesthetized dogs

| *Dose of GABA (mg)* | *Mean control temp. (°C ± s.e.)* | *Max. temp. rise (°C) after GABA (mean ± s.e.)* | *Latency of temp. response (min)* | *Time to max. temp. response (min)* | *Duration of temp. response (min)* |
|---|---|---|---|---|---|
| 0.1 (3) | 36.08 ± 0.2 | 0.5 ± 0.14 | 2-3 | 5-7 | 15-25 |
| 0.3 (4) | 36.18 ± 0.4 | 0.56 ± 0.06 | 2-3 | 5-7 | 40-60 |
| 1 (3) | 36.25 ± 0.38 | 0.25 ± 1.02 | 2-3 | 5-7 | 40-60 |
| 5 (3) | 37.83 ± 0.32 | 0.66 ± 0.25 | 2-3 | 5-7 | 40-80 |

Figures in parentheses indicate the number of experiments.

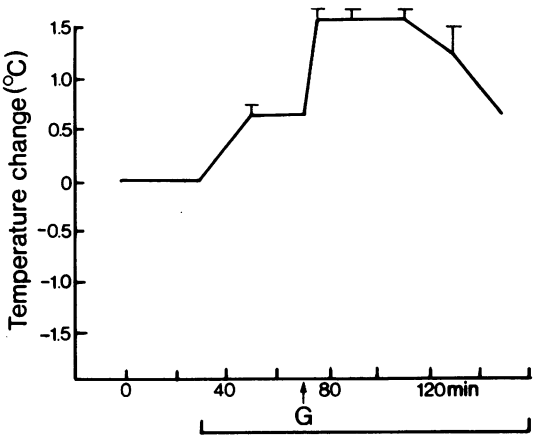


**Fig. 1** Record in dogs under intravenous pentobarbitone sodium (30 mg/kg) anaesthesia of the rectal temperature change during perfusion of the cerebral ventricles from left lateral ventricular to aqueductal cannulae with artificial CSF. At G, GABA, 5 mg, was injected intraventricularly. The bracket indicates the presence in the artificial CSF of BOL (4 μg/ml) and phentolamine (20 μg/ml). Vertical lines indicate s.e. of means (three experiments).

greater than those produced by the control effluent (four experiments). GABA (15 mg) and 0.25 ml of artificial CSF had no effect on the stomach strip. In three of the four experiments, BOL (75 μg) almost completely blocked the 5-hydroxytryptamine-induced contractions, but reduced the height of contractions due to control effluent and GABA effluent to the same extent (Figure 2). Presence of 5-hydroxytryptamine in the effluent confirms the findings of Feldberg & Myers (1966). In one experiment, the contractions produced by control effluent and those produced by GABA effluent were not blocked by BOL.

*Investigation of the possible release of acetylcholine into the effluent following intraventricular injection of GABA.* In these experiments the ventricles were perfused with artificial CSF containing physostigmine (3.3 μg/ml). This procedure did not affect rectal temperature, but the animals exhibited profuse salivation, twitching of the facial muscles and straightening of the ears. Acetylcholine, 50 and 100 ng, 0.1 ml of a 1/100 dilution of control effluent and 0.1 ml of a 1/100 dilution of GABA effluent (collected after 5 mg of GABA), all contracted the superfused guinea pig ileum. The contractions produced by GABA effluent were of the same height as those produced by the control effluent. Artificial CSF 0.1 ml and

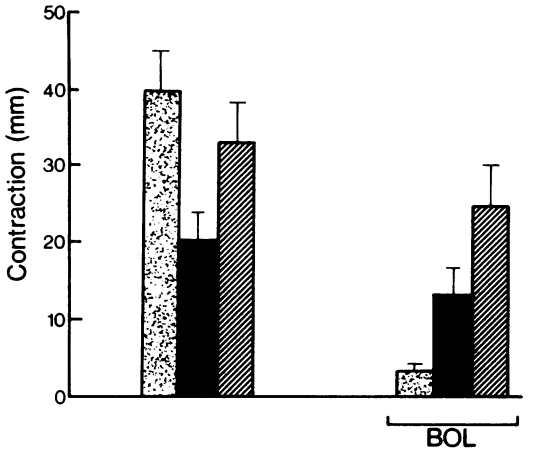


**Fig. 2** Rat isolated stomach strip bathed at 37° C with Krebs solution in a 15 ml organ bath (lever magnification, 15-fold). Contractile responses to 5 ng of 5-hydroxytryptamine, 0.25 ml of control effluent and 0.25 ml of GABA effluent are depicted by stippled, solid and diagonally hatched bars respectively. Bracket indicates the presence in the bath fluid of BOL (75 μg). Vertical lines indicate s.e. of means (three observations).

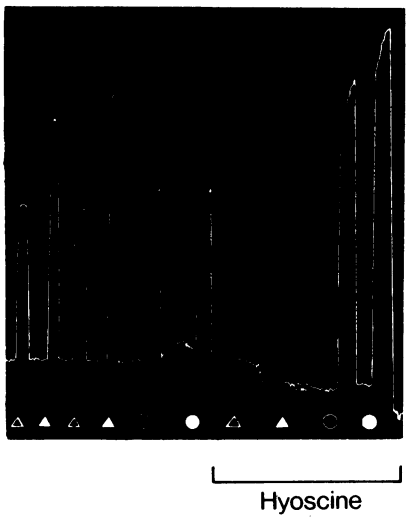


**Fig. 3** Guinea-pig isolated ileum superfused at 35° C with Tyrode solution (2 ml/min; lever magnification, 8-fold) containing hexamethonium (20 μg/ml), mepyramine (0.5 μg/ml) and morphine (1 μg/ml). Contractile responses to 50 ng of acetylcholine (△), 100 ng of acetylcholine (▲), 0.1 ml of a 1/100 dilution of control effluent (○) and 0.1 ml of a 1/100 dilution of GABA effluent (●). Bracket indicates the presence in the Tyrode solution of hyoscine (1 μg/ml).

GABA in a concentration of 1 mg/ml (the concentration possibly achieved in the effluent after injecting a 5 mg dose) diluted 1/100 produced a slight contraction. Contractions produced by acetylcholine were blocked by hyoscine (1 μg/ml), whereas those due to the control effluent and GABA effluent were potentiated (four experiments; Figure 3).

*Investigation of the possible mediation of the hyperthermic effect of intraventricular injection of GABA through the release of prostaglandin-like substances.* The possible release of prostaglandin-like substance was tested on the rat isolated stomach strip and rat isolated colon. Two hours after equilibration with antagonists, 0.25-0.5 ml of control effluent or GABA effluents, collected after the intraventricular injection of 0.1 and 0.3 mg doses of GABA, all contracted the stomach strip. The heights of the contractions produced by the GABA effluent following 0.1 mg of GABA were slightly greater than those produced by the control effluent, whereas those produced by the effluent immediately following the 0.3 mg dose were significantly ($P < 0.01$) greater. The second successive 20 min sample of effluent following the 0.3 mg dose was also more active than control ($P < 0.05$). The activity in the third sample, though still apparently greater than control, was not significantly so ($P > 0.05$) (three experiments, Figure 4). A fourth sample which was collected in one experiment was more active than control but less active than the third sample. Figure 4 also shows that there is a temporal correlation between the hyperthermic effect of GABA and its ability to release a substance capable of contracting the stomach strip. When 0.5 ml of the control effluent or GABA effluent (0.3 mg dose) was tested on the rat colon equilibrated for 1 h with antagonists, there was very little effect. $PGE_1$ was approximately 10 times less active on this preparation than on the stomach strip i.e. the height of contraction produced by 150 ng of $PGE_1$ matched that produced by 15 ng on the stomach strip.

In three other experiments in which 0.3 mg of GABA produced a mean rise in temperature of $0.5 \pm 0^{\circ}C$, 50 μg of $PGE_1$ injected intraventricularly had no effect on the temperature but 100 μg of $PGE_1$ produced a mean rise in temperature of $0.5 \pm 0^{\circ}C$, urination, defaecation and sometimes shivering. When the temperature had returned to normal, ventricular perfusion with sodium salicylate (250 μg/ml) was begun. GABA given 2 h after the start of perfusion now produced a fall in temperature ($0.8 \pm 0.03^{\circ}C$) but

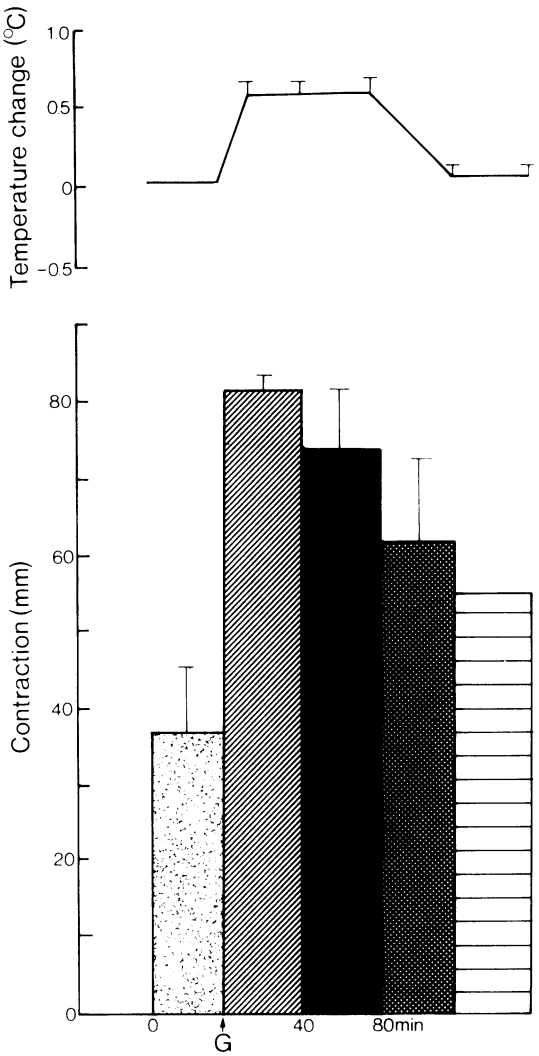


**Fig. 4** Record in dogs under intravenous pentobarbitone sodium (30 mg/kg) anaesthesia of the rectal temperature change during perfusion of the cerebral ventricles from left lateral ventricular to aqueductal cannulae with artificial CSF. Effluents from the perfused ventricles were collected in 20 min samples. The bars below the temperature record depict contractile responses of the rat isolated stomach strip to 0.25-0.5 ml of control effluent (stippled) and first (diagonally hatched), second (solid), third (cross-hatched) and fourth (horizontal) samples of GABA effluent. The stomach strips were bathed at 37° C with Krebs solution in 15 ml organ baths (lever magnification 15-fold) containing 1.0 μg each of BOL, mepyramine, hyoscine, phenoxybenzamine and propranolol. These drugs blocked the effects of histamine, 5-hydroxytryptamine, acetylcholine and catecholamines. At G, 0.3 mg GABA was injected intraventricularly. Vertical lines indicate s.e. of means (three observations).

100 μg of $PGE_1$ was as effective as before the start of salicylate perfusion (Figure 5). Control effluent collected 2 h following salicylate perfusion produced negligible contraction of the stomach strip. Similarly the activity of the GABA effluent collected during salicylate perfusion was significantly ($P < 0.05$) reduced (Figure 5). When the stomach strip was exposed to 0.5 ml of a solution containing 250 μg/ml of sodium salicylate, there was no effect. Two hours after discontinuing salicylate perfusion, 0.3 mg of GABA still produced a fall in temperature and the ability of control effluent and GABA effluent to contract the stomach strip continued to remain markedly diminished.

In the absence of salicylate and when assayed in terms of $PGE_1$, the control effluent contained 163 ± 35.4 ng/20 min and the GABA effluent, following the 0.3 mg dose, contained 460 ± 30 ng/20 min of prostaglandin-like substance (eight experiments). The activity in the GABA effluent was significantly greater ($P < 0.001$) than that in the control effluent.

*Investigation of the possible mediation of the hypothermic effect of intraventricular injection of GABA through the release of noradrenaline.* Beginning 1 h after the start of ventricular perfusion, the noradrenaline content of the 20 min effluent samples collected over a period of 6 h ranged between 120 ± 5.7-270 ± 17 ng (Figure 6). Thus, there was a continuous release of noradrenaline into the ventricles.

Table 1 shows that the hyperthermic effect of GABA was not dose-related. The noradrenaline content of the GABA effluents in these experiments was not significantly ($P > 0.1$) affected by the 0.1, 0.3 and 5 mg doses. With the 1 mg dose, however, the noradrenaline content was 648 ± 32.5 ng/20 min and this value was significantly greater ($P < 0.001$) than that in the control effluent.

Since sodium salicylate reversed the hyperthermic effect of 0.3 mg of GABA and markedly reduced the ability of the GABA effluent to contract the rat isolated stomach strip, experiments were made to determine whether reversal was associated with a release of noradrenaline into the effluent. Figure 7 illustrates that the hyperthermia (0.6 ± 0.06° C) elicited with a 0.3 mg dose of GABA was changed in the presence of salicylate to hypothermia and that there was a significantly ($P < 0.001$) increased release of noradrenaline into the ventricles.

In another series of experiments, the effects of different doses of GABA were compared with the hypothermia produced by noradrenaline, 500 ng.

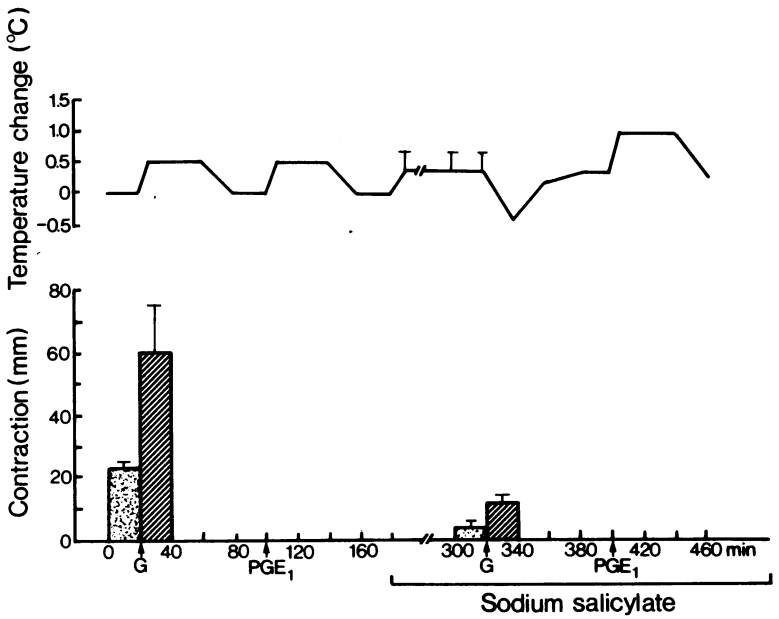


**Fig. 5** Record in dogs under intravenous pentobarbitone sodium (30 mg/kg) anaesthesia of the rectal temperature change during perfusion of the cerebral ventricles from left lateral ventricular to aqueductal cannulae with artificial CSF. Effluents from the perfused ventricles were collected in 20 min samples. The bars below the temperature record depict contractile responses of the isolated rat stomach strips (for details refer to Fig. 4) to 0.25-0.5 ml of control effluent (stippled) and GABA effluent (diagonally hatched). GABA effluent is defined as the effluent collected immediately after injecting intraventricularly 0.3 mg of GABA at G. At $PGE_1$, 100 $\mu$g of $PGE_1$ was injected into the ventricles. The bracket indicates the presence in the artificial CSF of sodium salicylate (250 $\mu$g/ml). Vertical lines indicate s.e. of means (three observations).

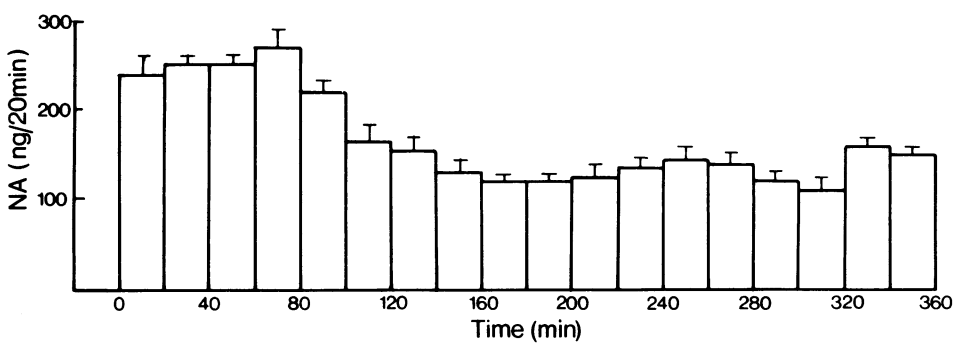


**Fig. 6** The bars indicate the noradrenaline content in ng of 20 min successive effluent samples collected from the aqueductal cannula during perfusion with artificial CSF of the cerebral ventricles from left lateral ventricular to aqueductal cannulae in dogs anaesthetized with intravenous pentobarbitone sodium (30 mg/kg). Vertical lines indicate s.e. of means (three observations).

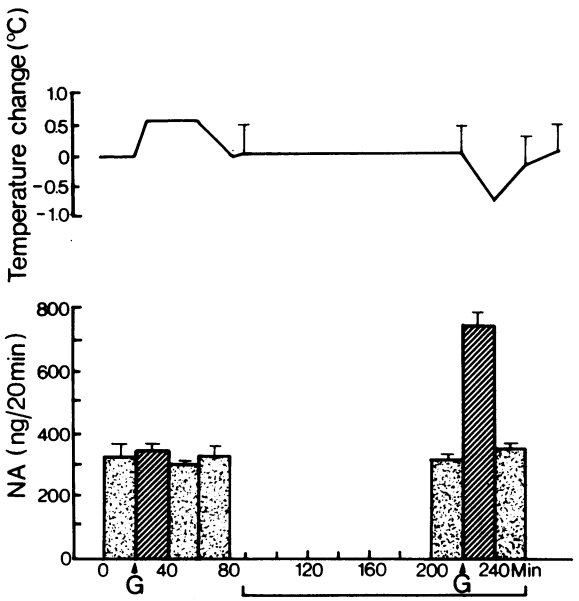


**Fig. 7** Record in dogs under intravenous pentobarbitone sodium (30 mg/kg) anaesthesia of the rectal temperature change during perfusion of the cerebral ventricles from left lateral ventricular to aqueductal cannulae with artificial CSF. Effluents from the perfused ventricles were collected in 20 min samples. The bars below the temperature record depict the noradrenaline content in ng of the control effluent (stippled) and GABA effluent (diagonal). GABA effluent is defined as the effluent collected immediately after injecting intraventricularly 0.3 mg of GABA at G. The bracket indicates the presence in the artificial CSF of sodium salicylate (250 μg/ml). Vertical lines indicate s.e. of means (four observations).

GABA, 0.1 mg, produced hyperthermia without any effect on the release of noradrenaline; GABA, 0.3 mg, produced less hyperthermia and a significantly ($P < 0.05$) increased release of noradrenaline whereas 1 and 5 mg produced hypothermia and significantly ($P < 0.01$) increased the release of noradrenaline, maximum effects being observed with the 1 mg dose (Figure 8). The 1 mg dose of GABA was therefore used in subsequent experiments.

The mean noradrenaline content of the effluent obtained from three reserpinized dogs was 132 ±12 ng/20 min and the mean rectal temperature was 34.9 ± 0.7°C (four experiments). The noradrenaline value was significantly ($P < 0.05$) lower than the respective control value shown in Figure 8a. Noradrenaline, 500 ng and GABA, 1 mg, produced hypothermia (0.5 ± 0.08°C and 0.8 ± 0.13°C respectively). GABA had no significant ($P > 0.1$) effect on the noradrenaline content of the effluent.

In three experiments, noradrenaline, 500 ng, and GABA, 1 mg, produced hypothermia (0.5 ± 0°C and 0.6 ± 0.13°C respectively) and GABA significantly ($P < 0.01$) increased the release of noradrenaline. Thirty minutes later, perfusion of the ventricles with phentolamine (20 μg/ml) was begun. This produced hyperthermia (0.7 ± 0.12°C) beginning within 5-8 min of the start of perfusion and continuing during the remaining period of perfusion. The noradrenaline content of the effluent was not affected. Noradrenaline now had no effect on temperature. GABA produced hyperthermia (0.6 ± 0.17°C) and had no significant effect ($P > 0.05$) on the noradrenaline content of the effluent.

In four experiments, the ventricles were perfused with calcium-free artificial CSF containing sodium edetate (0.37 mg/ml) for 1 h followed by perfusion with calcium-free artificial CSF. There was marked hypothermia (3.0 ± 0.67°C) within 70 min of the start of perfusion and the temperature remained depressed even though sodium edetate was omitted subsequently. This treatment did not affect the spontaneous release of noradrenaline into the ventricles. Noradrenaline, 500 ng, and GABA, 1 mg, had no effect on temperature. However, the release of noradrenaline following GABA was significantly ($P < 0.001$) less than the value in Figure 8c. The ventricles were then perfused with normal artificial cerebrospinal fluid. There was a restoration of the hypothermic action of noradrenaline, but neither the noradrenaline-releasing action of GABA nor its hypothermic action were restored.

## Discussion

GABA, 0.1, 0.3, 1 and 5 mg injected into the cerebral ventricles of anaesthetized dogs produced variable temperature effects; the 0.1 and 0.3 mg doses always produced hyperthermia, whereas the 1 and 5 mg doses produced either hyperthermia or hypothermia.

Intraventricularly or intrahypothalamically injected 5-hydroxytryptamine and acetylcholine produce hyperthermia (Feldberg *et al.*, 1967; Myers & Yaksh, 1969). However, the possibility that the GABA-induced hyperthermia is mediated by 5-hydroxytryptamine or acetylcholine is excluded because of (1) the increased hyperthermic response observed with GABA in dogs treated with reserpine which is known to deplete brain 5-hydroxytryptamine (Pletscher, Shore & Brodie, 1956); (2) the inability of BOL, which blocks brain tryptamine receptors (Tedeschi, Tedeschi &

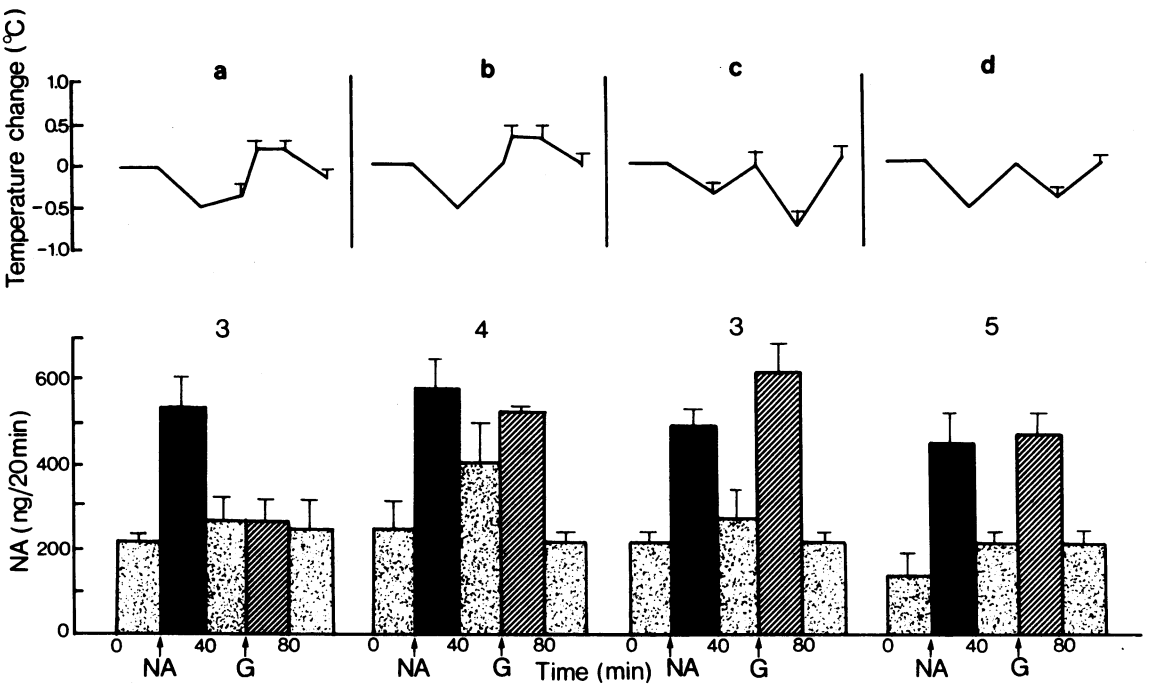


**Fig. 8** Records in dogs under intravenous pentobarbitone sodium (30 mg/kg) anaesthesia of the rectal temperature change during perfusion of the cerebral ventricles from left lateral ventricular to aqueductal cannulae with artificial CSF. Effluents from the perfused ventricles were collected in 20 min samples. The bars below the temperature records depict the noradrenaline content in ng of the control effluent (stippled), noradrenaline effluent (solid) and GABA effluent (diagonally hatched). Noradrenaline effluent is defined as the effluent collected immediately after injecting intraventricularly 500 ng noradrenaline at the point marked NA. GABA effluent is defined as the efluent collected immediately after injecting intraventricularly at G, 0.1 mg of GABA in (a), 0.3 mg of GABA in (b), 1 mg of GABA in (c) and 5 mg of GABA in (d). Vertical lines indicate s.e. of means. Figures above sets of columns indicate the numbers of observations.

Fellows, 1959), and of hyoscine, a drug closely allied to atropine which blocks the hyperthermic effects of intraventricularly injected carbachol (Hall, 1972), to antagonize GABA-induced hyperthermia; and (3) the failure of GABA to release 5-hydroxytryptamine or acetylcholine into the effluent.

When tested for the presence of prostaglandin-like substances on the isolated rat stomach strip, both the control effluent and the GABA effluent showed activity, the latter being much more potent. There was a temporal correlation between the hyperthermic effect of GABA and the release by it of prostaglandin-like substance (Figure 4). Intraventricular perfusion with sodium salicylate did not affect PGE-induced hyperthermia, but it blocked GABA-induced hyperthermia and significantly reduced the release of prostaglandin-like substances. Salicylates inhibit the prostaglandin synthetase system (Vane, 1971). Accordingly, GABA-induced hyperthermia could be interpreted in terms of release of prostaglandin. Although no categorical statement can be made as to the type of prostaglandin released by GABA, the lack of any significant effect of the effluents on the rat isolated colon would seem to suggest the release of prostaglandins of the E series, since the rat stomach strip and the rat colon can differentiate between prostaglandins of the E and F series (Gryglewski & Vane, 1972). Pertinent in the light of this suggestion are the results of Milton & Wendlandt (1971) who demonstrated a powerful hyperthermic effect of very small quantities of intraventricularly injected $PGE_1$ and $PGE_2$ and no effect of the prostaglandins of A and F series.

Holmes (1970) reported a total prostaglandin release of the order of 1-5 ng of $PGE_1$ equivalents/min from perfused dog cerebral ventricles. In our experiments the value was about 8 ng/minute. The difference may be ascribed to the differences in the methods of perfusion, since Holmes (1970) collected the effluent from the cisternal canula

and with this method the effluent contains an admixture of CSF (Feldberg, Myers & Veale, 1970).

Nanogram quantities of $PGE_1$ administered into the lateral ventricles of unanaesthetized cats with permanently implanted cannulae are hyperthermic (Feldberg & Saxena, 1971a). In our experiments with anaesthetized dogs much higher doses were needed. Moreover, in the anaesthetized dogs, GABA was also much less potent in producing hyperthermia than it was in unanaesthetized dogs. The low potency could at least partly be due to pentobarbitone anaesthesia, a suggestion entertained by Feldberg & Saxena (1971b). A continuous spillage of the intraventricularly injected drug into the effluent, inherent in our experimental technique, would make much less of the drug available for action. This could also account for the lesser potency of $PGE_1$ and GABA.

A continuous release of noradrenaline into the cerebral ventricles demonstrated in the present study ,supports the results of Phillipu, Heyd & Burger (1970). Hypothermia observed with GABA was associated with a significant release of noradrenaline into the ventricles. In some experiments, a significantly increased release of noradrenaline after GABA 0.3 mg and 1 mg, was associated with much less hyperthermia.

Dogs treated with reserpine were hypothermic confirming the hypothermic effect of reserpine reported in the literature for many species (Borison & Clark, 1967). The noradrenaline content of the effluent from reserpinized animals was significantly less than that of normal animals, implying depletion of noradrenaline stores. In these animals, GABA produced hyperthermia and no longer released noradrenaline, further supporting the role of noradrenaline release in mediating GABA-induced hypothermia. An unexplained result of these experiments was the production of hyperthermia by noradrenaline. During perfusion with phentolamine an $\alpha$-adrenoceptor blocking agent, noradrenaline-induced hypothermia was blocked and GABA-induced hypothermia was converted to hyperthermia. This result is in accord with the reported hypothermic effect of intraventricularly injected or endogenously released noradrenaline, being mediated by $\alpha$-adrenoceptors (Brittain & Handley, 1967; Burks, 1971; Feldberg & Saxena, 1971c).

Calcium is necessary for the release of noradrenaline from nerve endings and hypothalamus (Boullin, 1965; Phillipu *et al.*, 1970). It, therefore, seemed interesting to examine if the release of noradrenaline by GABA was calcium-dependant. Intraventricular perfusion with calcium-free solution and sodium edetate (which chelates calcium) was associated with marked hypothermia. This result is apparently, contrary to what is reported by Myers & Veale (1971) and Myers & Yaksh (1971) for cats and monkeys and is the subject of another report (Dhumal & Gulati, 1973). During perfusion with calcium-free solution, the spontaneous release of noradrenaline was not affected, but GABA failed to release noradrenaline or have any effect on temperature. In contrast with experiments with reserpine and phentolamine, in these experiments there was no unmasking of the hyperthermic effect of GABA. Thus it would appear that the release of both noradrenaline and PGE-like substance by GABA is calcium-mediated. The effects of GABA were not restored during perfusion with solution containing calcium, perhaps due to incomplete restitution of the calcium stores. Failure of noradrenaline to cause hypothermia in these experiments, coupled with the restoration of the effect of noradrenaline during perfusion with a calcium-containing solution, would suggest a role for calcium in the mediation of noradrenaline-induced hypothermia.

$PGE_1$ and $PGE_2$ are released from the rabbit heart in response to sympathetic nerve stimulation, apparently in amounts that can inhibit the secretion of noradrenaline from the neurone. When the synthesis of PGE is blocked by 5, 8, 11, 14-eicosatetraynoic acid, the output of noradrenaline is increased (Wennmalm, 1971). Thus, prostaglandins of the E type serve as an endogenous 'braking' mechanism for controlling the secretion of noradrenaline (Hedqvist, 1969). In the present study, the administration of sodium salicylate, which is a blocker of prostaglandin synthetase (Vane, 1971), led to an increased release of noradrenaline by GABA and conversion of GABA-induced hyperthermia to hypothermia. In this respect, the effects of GABA are analogous to those of sympathetic nerve stimulation.

Since the hypothalamus contains noradrenaline (Vogt, 1954), $PGE_1$ (Holmes & Horton, 1968) and GABA (Berl & Waelsh, 1958), it would be tempting to speculate that GABA is intimately concerned with temperature regulation through the release of $PGE_1$ and noradrenaline. $PGE_1$ is hyperthermic in nanogram quantities only when injected into the anterior hypothalamus and it has been suggested that prostaglandins are continuously released to maintain body temperature (Feldberg & Saxena, 1971b). Presumably, endogenous GABA in the anterior hypothalamus effects a continuous release of $PGE_1$. According to this scheme, the release of a hypothermic substance (noradrenaline in this case) would be necessary to overcome the excessive hyperthermia produced by $PGE_1$.

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