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DISSIMILAR EFFECTS ON BODY TEMPERATURE IN THE CAT PRODUCED BY GUANOSINE 3′,5′-MONOPHOSPHATE, ACETYLCHOLINE AND BACTERIAL ENDOTOXIN

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1 Guanosine 3′,5′-monophosphate (cyclic GMP) and N^2-2′-O-dibutyryl guanosine 3′,5′-monophosphate (db cyclic GMP) have been injected into the third cerebral ventricle (i.c.v.) of the unanaesthetized cat and the effects on rectal temperature and on behavioural and autonomic activities observed and compared with those of acetylcholine and physostigmine.

2 Acetylcholine (100 nmol) and physostigmine (100 nmol) injected together i.c.v. produced a rise in body temperature in cats at an environmental temperature of 20–24°C, which was abolished by pretreatment i.c.v. with atropine (200 nmol).

3 Cyclic GMP and db cyclic GMP (10–1250 nmol) had no effect on body temperature in cats at an environmental temperature of 20–24°C but produced hypothermia (1250 nmol) in cats at an environmental temperature of 9–11°C.

4 The *O*-somatic antigen of *Shigella dysenteriae* (20 μg/kg i.v.) produced fever in cats which was not potentiated by caffeine (25 mg/kg i.p.). Levels of endogenous cyclic GMP in c.s.f. taken from the cisterna magna during fever induced by bacterial endotoxin in the presence or absence of paracetamol (50 mg/kg i.p.) and/or caffeine were similar to values for afebrile cats.

5 It is concluded that exogenous cyclic GMP and db cyclic GMP can inhibit central events mediating autonomic and behavioural thermoregulation stimulated in cats by exposure to cold environments.

Introduction

Acetylcholine (ACh) is a putative neurotransmitter in central thermoregulatory pathways mediating both heat loss and heat gain (see reviews by Brimblecombe, 1973; Feldberg, 1975; Milton, 1978). Central cholinoceptive neurones regulating heat loss in the cat are 'nicotinic' and those controlling heat gain are 'muscarinic' (Hall, 1972; 1973). The intracellular events mediating changes in neuronal activity caused by receptor binding are poorly understood but those subsequent to muscarinic receptor-agonist binding are thought to include raised concentrations of guanosine 3′,5′-monophosphate (cyclic GMP) (Ferrendelli, Steiner, McDougal & Kipnis, 1970; Kuo, Lee, Reyes, Walton, Donnelly & Greengard, 1972; Lee, Kuo & Greengard, 1972; Kebabian, Bloom, Steiner & Greengard, 1975; Palmer & Dusynski, 1975). It is possible that cyclic GMP may be involved in central muscarinic neurones regulating heat production/conservation, a hypothesis supported by the observation that concentrations of cyclic GMP are increased in the hypothalamus and the brainstem of the cold-exposed rat (Mao, Guidotti & Costa, 1974).

Robison, Butcher & Sutherland (1971) stated four criteria, together with some of their limitations, which are essential in substantiating the involvement of adenosine 3′,5′-monophosphate (cyclic AMP) in a hormonal response. Briefly, these are the ability of the hormone to stimulate cyclic AMP production in broken cell preparations, for exogenous cyclic AMP to mimic the effect of the hormone, for the hormone to increase intracellular cyclic AMP in intact cells and for phosphodiesterase inhibitors to increase the magnitude of the hormone response. Due to a number of chemical, metabolic and biological similarities between the cyclic AMP and cyclic GMP systems, similar criteria have been employed in most investigations concerning cyclic GMP (see reviews by Goldberg, O'Dea & Haddox, 1973; Goldberg & Haddox, 1977; Greengard, 1978; 1979). We have investigated the possible involvement of cyclic GMP in central thermoregulatory pathways in the conscious cat by applying the latter three criteria, i.e. by comparing the thermoregulatory response to cyclic GMP

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with that of acetylcholine plus physostigmine (ACh + Phys), by measuring endogenous cyclic GMP during fever, and by studying the effect of caffeine, a phosphodiesterase inhibitor, on body temperature in normal and febrile cats. A preliminary account of part of this work has been communicated to the British Pharmacological Society (Dascombe & Milton, 1980).

Methods

Female cats weighing from 1.8 to 3.6 kg were used. Animals were caged individually during experiments and body temperature, measured with a Yellow Springs Instrument 401 thermistor inserted about 10 cm into the rectum, was monitored continuously. Experiments were conducted at approximately the same time each day at an environmental temperature of 20–24°C unless otherwise indicated.

Micro-injection studies

Under aseptic conditions and during pentobarbitone sodium (40 mg/kg i.p.) anaesthesia, a guide cannula (21-gauge needle tubing) was implanted into the third cerebral ventricle with its tip at the level of the preoptic and anterior hypothalamic nuclei. The coordinates were taken from the stereotaxic atlas of the cat brain by Snider & Niemer (1961). Cats were allowed to recover for at least one week before being used in experiments.

Drugs were dissolved in sterile, pyrogen-free 0.9% w/v NaCl solution (Steriflex, Allen & Hanburys Ltd) and injected into the third cerebral ventricle (i.c.v.) through an injection cannula (26-gauge needle tubing) passed through the previously implanted guide cannula to a site 1 mm beyond the tip of the outer tube. The volume of fluid injected over a period of 40–60 s was 50 μl. Aseptic injection procedure was used as previously described (Dascombe & Milton, 1975a). Each animal was used at intervals of not less than 72 h.

Upon completion of experiments, the injection site in each cat was verified *post mortem* (Dascombe & Milton, 1975a).

Cyclic GMP in c.s.f.

Samples of c.s.f. were obtained from the cisterna magna of the unanaesthetized cat by the method of Feldberg, Gupta, Milton & Wendlandt (1973). Cats were allowed to recover for at least two weeks after implantation of the cisternal guide cannula before being used in experiments. Each animal was used at intervals of about 7 days.

The *O*-somatic antigen of *Shigella dysenteriae* 20 μg/kg (Humphrey & Bangham, 1959) was injected intravenously into cats. The minimum period between two injections of endotoxin into any one animal was 7 days to minimize the development of tolerance to the endotoxin. Paracetamol (50 mg/kg) and caffeine (25 mg/kg) were injected intraperitoneally. Drug solutions were passed through a Millex filter (pore size 220 nm) before administration.

Samples of c.s.f. (0.3–0.6 ml in volume) were collected at the times indicated in Results into 10 μl 0.25 M disodium edetate (EDTA) and stored in sealed ampoules at −20°C in the dark until assay. Samples were deproteinized (Dascombe & Milton, 1976) and the concentration of cyclic GMP measured by radio-immunoassay (Radiochemical Centre Ltd).

Statistical analysis

Temperature responses were assessed as a thermal response index (TRI) integrating the change in body temperature (°C) against duration (h) such that a single TRI unit (TRI 1°C.h) is equivalent to a 1°C change in temperature lasting 1 h. The value of x in TRI_x is the period of time (h) for which the response has been assessed. Results are expressed as the mean ± s.e.mean for n experiments. The probability (P) of the significance of the difference between different groups was determined by a t test for related data (Armitage, 1971).

Materials used

The following drugs were used: acetylcholine chloride, atropine sulphate, caffeine, physostigmine sulphate, sodium n-butyrate (BDH Chemicals Ltd); guanosine 3′,5′-monophosphoric acid sodium salt, guanosine 5′-monophosphoric acid sodium salt, N^2-2′-O-dibutyryl guanosine 3′,5′-monophosphoric acid sodium salt (Sigma Chemical Co.); paracetamol (Koch Light). Reagents for cyclic GMP assay (Radiochemical Centre, Amersham, England).

Results

Thermoregulatory effects of acetylcholine and physostigmine

ACh + Phys (100 nmol + 100 nmol i.c.v.) caused hyperthermia in cats (Figure 1) concomitant with piloerection, shivering and ear skin vasoconstriction beginning within 5 min of injection although peripheral vasodilatation was seen in one cat. ACh + Phys induced mewing and flattening of the pinnae in some animals. Atropine (200 nmol), administered i.c.v. 15 min before ACh + Phys, abolished the hyperthermia and autonomic responses to the

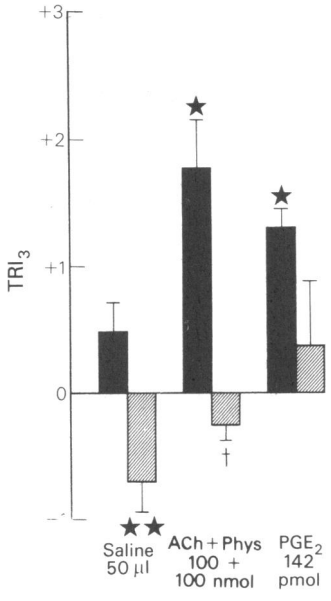

Figure 1 Effects of acetylcholine plus physostigmine (ACh + Phys) and prostaglandin E_2 (PGE_2) on rectal temperature in cats at an environmental temperature of 20–24°C. Columns represent the mean thermal response index for 3 h (TRI_3), vertical lines show s.e.mean ($n = 5$). Drugs injected i.c.v. 15 min after saline (50 µl solid columns) or atropine (200 nmol, hatched columns). The significance of the difference from the saline plus saline response is presented as $*0.05 > P > 0.01$; $**0.01 P > P > 0.001$ and from the saline plus (ACh + Phys) response as $\dagger 0.01 > P > 0.001$.

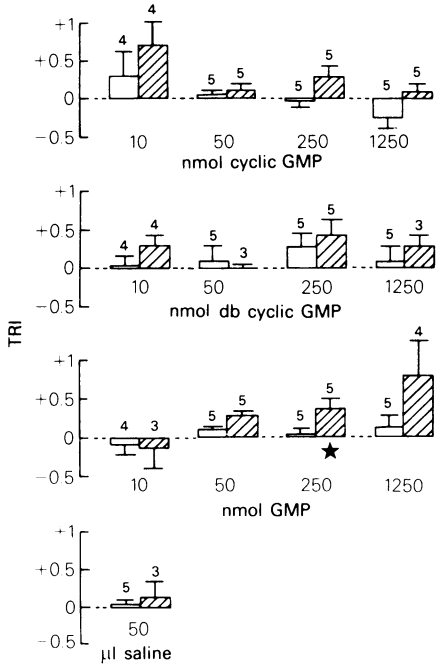

Figure 2 Effect of guanine nucleotides on rectal temperature in cats at an environmental temperature of 20–24°C. Columns represent the mean thermal response index (TRI) for the time 0 to 2.5 h (open columns) and 2.5 to 5 h (hatched columns) after injection i.c.v. Vertical lines show s.e.mean. The number of experiments is indicated above the columns. The significance of the difference from the value for saline (50 µl) is shown as * for $0.05 > P > 0.01$.

cholinomimetic. Atropine alone caused a fall in body temperature (Figure 1) associated with tachypnoea, panting and cutaneous vasodilatation with onset about 5 min after injection. Prostaglandin E_2 (PGE_2) (142 pmol) injected i.c.v. in these cats elicited a rise in body temperature (Figure 1) together with ear skin vasoconstriction, shivering and a crouched posture which reduced the surface area: body mass ratio. The febrile response to PGE_2 was attenuated but not abolished by atropine pretreatment (Figure 1).

Thermoregulatory effects of guanine nucleotides

Cyclic GMP and db cyclic GMP (10, 50, 250 and 1250 nmol) did not produce any effect on body temperature in cats at an environmental temperature of 20–24°C over a period of 5 h after injection i.c.v. of the nucleotide (Figure 2). This lack of effect on body temperature was despite the production of ear skin vasodilatation and sweating from the foot pads following the administration of either compound. This autonomic heat loss activity had its onset 4–10 min after drug administration and lasted 40–60 min, being most pronounced after 1250 nmol db cyclic GMP which induced tachypnoea and also panting in some cats. Db cyclic GMP caused non-thermoregulatory effects including defaecation, excessive salivation, increased grooming activity and intermittent mewing during the period 2–40 min after injection. In contrast with the effects of db cyclic GMP, quiescence lasting 40–60 min was the principle behavioural response to cyclic GMP. Injection i.c.v. of GMP (10–1250 nmol) was without effect on body temperature (Figure 2), behaviour and autonomic activity up to 2.5 h after injection when compared with responses to saline (50 µl 0.9% w/v NaCl solution).

Cats responded to high doses (250 and 1250 nmol) of GMP with a sustained rise in temperature 2.5–5 h after injection of the nucleotide.

At an environmental temperature of 9–11°C cyclic GMP (1250 nmol) and db cyclic GMP (1250 nmol) produced a fall in body temperature beginning within 10 min after injection and lasting about 2.5 h (Figure 3). The development of the hypothermia induced by

cyclic GMP ($TRI_{2.5}$ − 1.24 ± s.e.mean 0.25°C.h, $n = 5$, $P < 0.01$) and by db cyclic GMP ($TRI_{2.5}$ − 1.26 ± 0.18°C.h, $n = 5$, $P < 0.01$) was associated with cessation of cold-induced shivering, piloerection and vasoconstriction, and the onset of cutaneous vasodilatation and, with db cyclic GMP, tachypnoea. The return of body temperature to pre-injection values was associated with the redevelopment of shivering, piloerection and cutaneous vasoconstriction in the cold-exposed cats. No other effect on body temperature or thermoregulatory activity was apparent in cats up to 5 h after injection of cyclic nucleotides when compared with responses to saline (50 μl i.c.v.). Cyclic GMP (250 nmol), GMP (1250 nmol) and sodium *n*-butyrate (2500 nmol) did not have a hypothermic effect on cold-exposed cats. GMP caused a sustained rise in body temperature (Figure 3) which was significantly different ($P < 0.02$) from the saline response 2.5–5 h after injection.

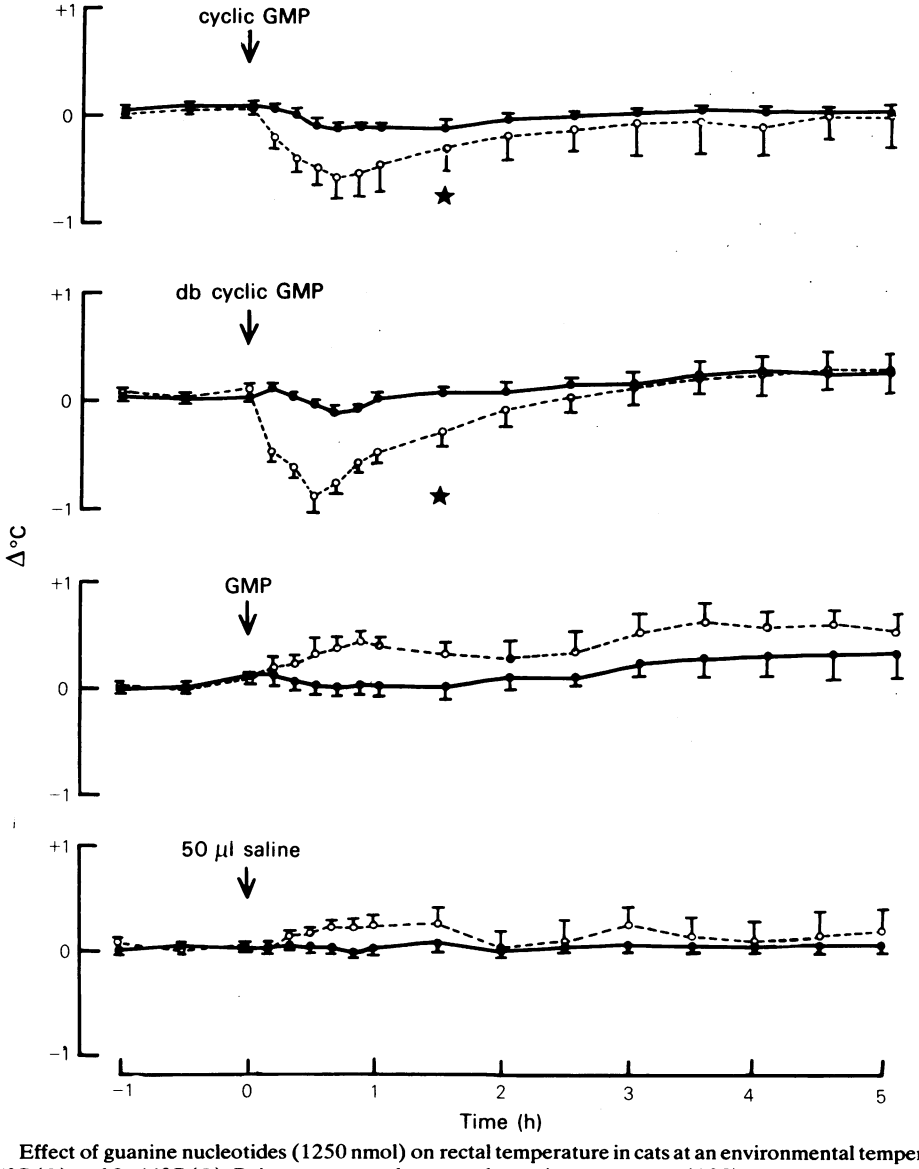

Figure 3 Effect of guanine nucleotides (1250 nmol) on rectal temperature in cats at an environmental temperature of 20–24°C (●) and 9–11°C (○). Points represent the mean change in temperature (Δ°C), vertical lines show s.d. of an observation ($n = 5$). The significance of the difference from the value for saline (50 μl) is $*0.01 > P > 0.001$ for the period 0 to 2.5 h.

Cyclic GMP in c.s.f.

The *O*-somatic antigen of *Shigella dysenteriae* (20 μg/kg i.v.) induced a biphasic rise in body temperature associated with ear skin vasoconstriction shivering and the adoption of a crouched posture reducing the surface area:body mass ratio. Febrile cats were more quiescent than control animals, appearing sedated and moving only after being disturbed. Fever was reduced by paracetamol (50 mg/kg i.p.) heat loss being effected mainly by peripheral vasodilatation (Figure 4a). Samples of c.s.f. obtained

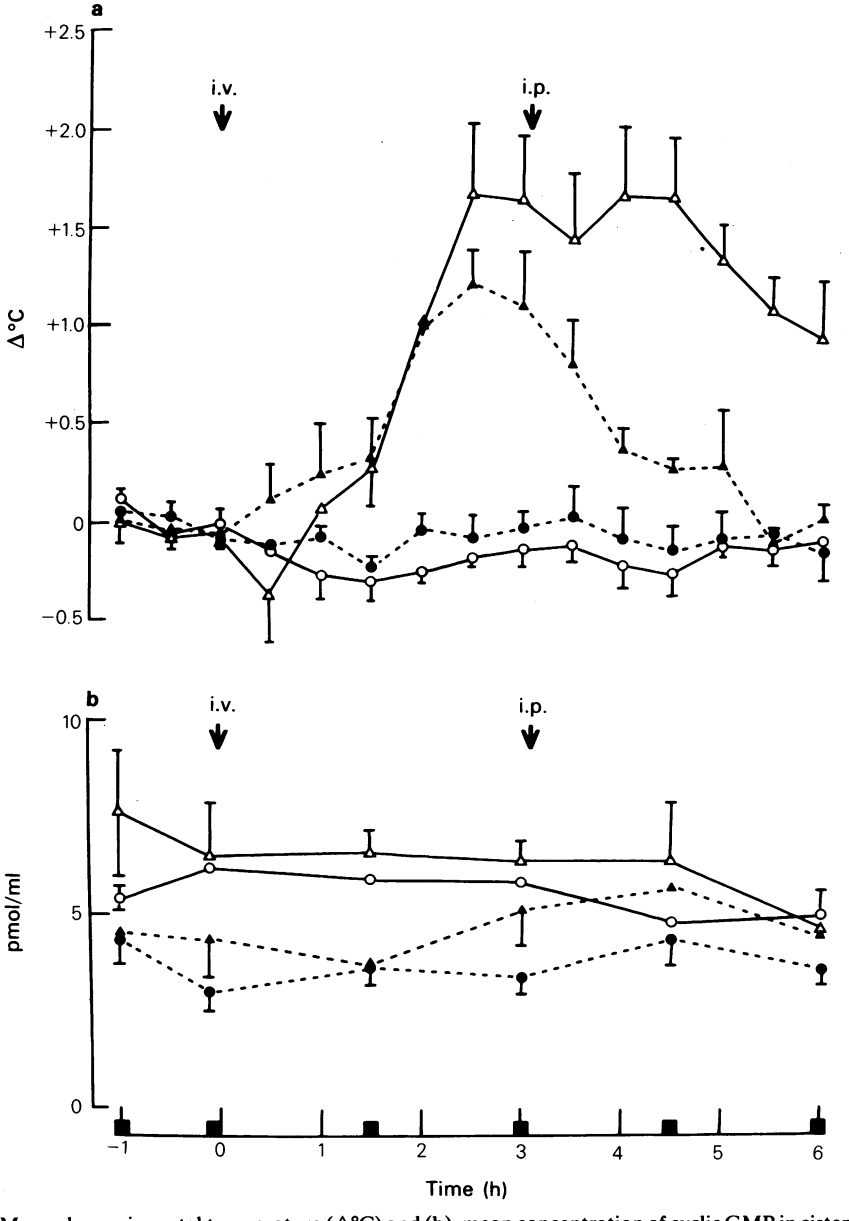

Figure 4 (a) Mean change in rectal temperature (Δ°C) and (b), mean concentration of cyclic GMP in cisternal c.s.f. (pmol/ml) in cats ($n = 3$). At the first arrow (i.v.) saline or *Shigella dysenteriae* 20 μg/kg was injected into the animals. At the second arrow (i.p.) propylene glycol/saline or paracetamol (50 mg/kg) was injected. Vertical lines define s.e.mean, overlapping values not always shown, and closed columns the time and duration of collecting c.s.f. samples. (○) Saline + glycol/saline; (●) Saline + paracetamol; (△) *S.dysenteriae* + glycol/saline; (▲) *S.dysenteriae* + paracetamol.

at the times indicated in Figure 4a, during the febrile and antipyretic phases, did not contain amounts of cyclic GMP different from those in the c.s.f. of cats receiving injections of vehicles (Figure 4b).

Caffeine (25 mg/kg) caused a rise in body temperature in cats (TRI_6 2.41 ± 0.36°C.h, $n = 3$, $P < 0.05$) which was associated with increased locomotor activity, the animals prowling continuously around their cages and showing increased alertness by responding more rapidly than control cats to auditory and visual stimuli. The rise in temperature induced by caffeine (25 mg/kg i.p.) injected 15 min before bacterial pyrogen partially added to the febrile response to the endotoxin (control pyrogen fever TRI_6 6.94 ± 1.26°C.h; fever in caffeine-treated cats TRI_6 7.96 ± 1.69°C.h, $n = 3$, $P < 0.05$). The behavioural and autonomic activities observed in cats following administration of bacterial pyrogen were unaffected by the presence of caffeine. Cyclic GMP levels in the c.s.f. of cats injected with caffeine were not significantly different from the control values shown in Figure 4b and were similar also after injection of *Shigella dysenteriae* endotoxin (20 μg/kg i.v.) and/or paracetamol (50 mg/kg i.p.).

Discussion

The results of this study are in agreement with earlier reports that cholinoceptive neurones regulate heat gain in the cat and are muscarinic (Hall, 1972; 1973). Hyperthermia was induced in cats of this study by ACh + Phys injected i.c.v., this route of administration being used because of the diffuse distribution of cholinoceptive neurones modulating heat gain (Myers & Yaksh, 1969). The response to ACh + Phys was abolished by atropine in contrast with the partial reduction of PGE_2-induced hyperthermia which was apparently a result of functional antagonism; PGE_2 increased heat gain and atropine promoted heat loss.

Cholinomimetics increase cyclic GMP concentrations in brain tissue (Ferrendelli *et al.*, 1970; Kuo *et al.*, 1972) by acting on muscarinic receptors (Lee *et al.*, 1972; Palmer & Dusynski, 1975). The hypothesis is therefore that the hyperthermia produced in cats by ACh + Phys and inhibited by atropine is mediated by muscarinic receptors and increased levels of endogenous cyclic GMP. However, exogenous cyclic GMP injected i.c.v., had no effect on body temperature unlike ACh + Phys in cats at an ambient temperature of 20–24°C, but induced some autonomic heat loss activity. It is possible that the lack of effect on body temperature of cyclic GMP was due to insufficient nucleotide entering neurones because of rapid hydrolysis by phosphodiesterases (Beavo, Hardman & Sutherland, 1970) and/or poor penetration across cell membranes (Posternak, 1971). Certain derivatives of cyclic nucleotides such as N^2-2′-O-dibutyryl guanosine 3′,5′-monophosphate (db cyclic GMP) and N^6-2′-O-dibutyryl adenosine 3′,5′-monophosphate (db cyclic AMP) are more resistant to hydrolysis and more lipid soluble than the parent nucleotides (Posternak, Sutherland & Henion, 1962; Posternak, 1971) and when applied extracellularly can act as intracellular sources of cyclic nucleotide and/or biologically active metabolites (Henion, Sutherland & Posternak, 1967; Kaukel & Hilz, 1972; Neelon & Birch, 1973). The activities of exogenous cyclic GMP and its derivatives on intact cell systems *in vivo* and *in vitro*, including their ability to mimic certain effects of ACh, have been reviewed (Hardman, 1971; Simon, Shuman & Robins, 1973; Goldberg & Haddox, 1977; Greengard, 1978). However, db cyclic GMP was (like cyclic GMP) without effect on body temperature in cats at an ambient temperature of 20–24°C although autonomic heat loss activity was induced. These observations indicate that endogenous cyclic GMP may regulate heat loss not heat gain in cats. Hypothermia produced in cats by cyclic GMP and db cyclic GMP injected i.c.v. has been reported (Clark, 1978) but only in doses of 5 mg and 1–2 mg respectively, and was followed by a rise in temperature lasting about 12 h. Increasing the dose of drug to this magnitude increases the probability of damaging tissue near the injection site and causing increased production and release of prostaglandin-like substances which, in the hypothalamus in the cat, cause a febrile response unrelated to the drug administered (Dey, Feldberg, Gupta, Milton & Wendlandt, 1974; Dascombe & Milton, 1975a). That cyclic GMP and db cyclic GMP promote heat loss was substantiated in this study by the nucleotides, in doses smaller than those used by Clark (1978), causing hypothermia in cats at an environmental temperature of 9–11°C. Cats exposed to the cold responded with autonomic and behavioural heat gain activity which was inhibited by cyclic GMP and db cyclic GMP. No sustained hyperthermia of late onset was observed in these cats. The effects of cyclic GMP and db cyclic GMP on body temperature are qualitatively similar to those of cyclic AMP and db cyclic AMP (Varagić & Beleslin, 1973; Clark, Cumby & Davis, 1974; Dascombe & Milton, 1975a). This similarity could be due to high concentrations of exogenous cyclic GMP activating cyclic AMP-dependent protein kinase activity (Goldberg *et al.*, 1973). Alternatively, the observation that cyclic GMP was a hypothermic agent like db cyclic GMP, despite cyclic GMP being less able than its dibutyryl derivative to cross cell membranes (Posternak, 1971), indicates that the responses to exogenous guanine nucleotides may be consequences of extracellular effects. Hypothermia may not reflect,

therefore, a role of increased endogenous cyclic GMP in the cat brain.

The second criterion tested was the association of heat gain activity with increased brain levels of endogenous cyclic GMP. Attempts to minimize *post mortem* rises in cyclic nucleotides in brain tissue (Breckenridge, 1964; Kakiuchi & Rall, 1968) have concentrated on methods unlikely to work satisfactorily with brain tissue in the cat (Schmidt, Schmidt & Robison, 1971; Lust, Passonneau & Veech, 1973; Nahorski & Rogers, 1973; Jones, Medina, Ross & Stavinoha, 1974). Cyclic GMP in c.s.f. may be derived in part from brain tissue as a result of cellular extrusion, a means by which intracellular concentrations of the nucleotide can be regulated (Goldberg *et al.*, 1973; Cramer, 1977). In these experiments c.s.f. levels of the nucleotide were measured during fever induced by a bacterial pyrogen when cholinergic heat gain pathways are presumed to be active for several hours. Attempts to correlate changes in c.s.f. cyclic GMP with those in brain tissue are limited to species convenient for present techniques for rapid fixation of brain tissue. In the rat, for example, ethanol causes a fall in cyclic GMP levels in both the brain (Hunt, Redos, Dalton & Catravas, 1977; Volicer & Hurter, 1977) and the c.s.f. (Weitbrecht & Cramer, 1980). Similar studies appear not to have been conducted for the cat but changes in c.s.f. levels of cyclic GMP have been reported (Sakai, Ary, Hymson & Shapiro, 1979). The *O*-somatic antigen of *Shigella dysenteriae* injected intravenously caused hyperthermia in cats as a result of increased autonomic and behavioural heat gain activity but c.s.f. levels of cyclic GMP were unaffected. It is conceivable that increased concentrations of cyclic GMP in thermoregulatory neurones do not cause changes in c.s.f. cyclic GMP due to metabolism of the nucleotide by phosphodiesterases but after administration of the phosphodiesterase inhibitor caffeine, endotoxin-induced hyperthermia was still without effect on c.s.f. cyclic GMP. These data contrast with values for c.s.f. cyclic AMP which under similar conditions have been found to increase (Dascombe & Milton, 1975b; 1976).

Potentiation of a response by a phosphodiesterase inhibitor is supportive evidence for the response being mediated by cyclic nucleotides (Butcher, 1968; Robison *et al.*, 1971) but no potentiation of the febrile response to bacterial pyrogen by caffeine was observed in agreement with earlier findings (Dascombe, 1977).

When assessed by the three criteria for cyclic GMP involvement applied in this study (see Introduction), the corroborative evidence of these results indicates that cyclic GMP in the brain does not mediate hyperthermia induced in cats by ACh + Phys or bacterial pyrogen. This interpretation must be regarded as provisional and awaits verification by data afforded by more discriminative techniques than those currently available to assess brain function in conscious animals. It is concluded that cyclic GMP and db cyclic GMP injected i.c.v. produce a fall in rectal temperature by inhibiting central events mediating heat conservation and production in cats exposed to cold environments.

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