Br. J. Pharmac. (1980), **70**, 453–459

THERMOREGULATORY EFFECTS OF N^6-2′-*O*-DIBUTYRYL ADENOSINE 3′,5′-MONOPHOSPHATE IN THE RESTRAINED MOUSE

M.J. DASCOMBE[1], A.S. MILTON, I. NYEMITEI-ADDO & R.G. PERTWEE

Department of Pharmacology, Marischal College, University of Aberdeen, Aberdeen, AB9 1AS

1 The N^6-2′-*O*-dibutyryl derivative of adenosine 3′,5′-monophosphate (db cyclic AMP) has been micro-injected into the third cerebral ventricle of the unanaesthetized, restrained mouse and the effects on body temperature and thermoregulatory activities observed.

2 Db cyclic AMP (4, 16 and 32 μg) injected intracerebroventricularly produced hypothermia when compared with temperature responses to sodium *n*-butyrate (6.8 μg).

3 Hypothermia induced by db cyclic AMP in mice was associated with a fall in oxygen consumption together with behavioural and autonomic heat loss activities but not cutaneous vasodilatation. The effects on rectal temperature and oxygen consumption were dose-dependent.

4 The falls in rectal temperature and oxygen consumption induced by db cyclic AMP (4 μg) were decreased by elevation of the environmental temperature from 22 to 32°C and abolished at 36°C.

5 It is concluded db cyclic AMP may inhibit central events mediating the rise in metabolic heat production in mice upon exposure to cold environments.

Introduction

Adenosine 3′,5′-monophosphate (cyclic AMP) is accepted as a modulator of activity in many types of cell and tissue including those of the mammalian central nervous system (for reviews see Drummond & Ma, 1975; Kebabian, 1977; Nathanson, 1977). However, characterization of the cyclic AMP system has depended extensively on the use of tissues *in vitro*. Attempts to determine whether raised concentrations of cyclic AMP have an effect *in vivo* equivalent to that anticipated from studies *in vitro* have included the administration of N^6-2′-*O*-dibutyryl adenosine 3′,5′-monophosphate (db cyclic AMP) to whole animals. Db cyclic AMP is more resistant than cyclic AMP to hydrolysis catalysed by cyclic nucleotide phosphodiesterases and may penetrate cell membranes more readily than the parent nucleotide (Posternak, Sutherland & Henion, 1962; Henion, Sutherland & Posternak, 1967).

The effects of central injections of db cyclic AMP on body temperature in conscious homeotherms could indicate an involvement of cyclic AMP in the central nervous system during thermoregulation. In this study, the effects of db cyclic AMP on body temperature in restrained, conscious mice have been studied after micro-injection of the drug into the third cerebral ventricle. Skin temperature as an index of peripheral vasomotor tone and oxygen consumption as an index of metabolic heat production have also been measured in order to identify the effector system mediating changes in body temperature.

Methods

Adult male albino mice (MF1 strain) were used. Changes in the rate of heat production were detected indirectly by the measurement of oxygen consumption rate (V_{O_2}). This was measured over a 60 to 90 min period by the closed-circuit system described by Pertwee & Tavendale (1977) and was expressed as volume of oxygen consumed per 25 g body weight per hour (ml 25 $g^{-1}h^{-1}$) after adjustment for s.t.p. Mice were held in a restraining apparatus (Pertwee, 1970; 1974). Deep body temperature was monitored with a thermistor probe (YSI 402) inserted 3 cm into the rectum. Paw skin temperature was measured by taping a thermistor (YSI 409) to the plantar surface of a hindpaw because measurement of paw temperature can provide an index of change in peripheral vasomotor tone (Pertwee, 1970), changes in which reflect changes in heat loss. Experiments were carried out at ambient temperatures of 22, 32 and 36°C. All temperatures were continuously monitored.

Injections were made into the IIIrd ventricle of the brain in volumes of 0.5 μl over a period of 1 min.

[1] Present address: Department of Pharmacology, Materia Medica and Therapeutics, Stopford Building, University of Manchester, Oxford Road, Manchester, M13 9PT

0007-1188/80/110453-07 $01.00

They were made through cannulae which had been implanted at least 5 days earlier into the skulls of mice weighing 19 to 21 g. The construction and stereotaxic placement of cannulae as well as the techniques both for injection and for subsequent verification of the injection site have been described elsewhere (Nyemitei-Addo, Pertwee & Tavendale, 1980).

N^6-2′-*O*-dibutyryl adenosine 3′,5′-monophosphate, monosodium salt (db cyclic AMP) was injected in a 0.9% w/v NaCl solution (saline). Control injections were made with solutions of sodium *n*-butyrate in saline having molarities equal or greater than the db cyclic AMP solutions used. Mice were given injections both of db cyclic AMP and of sodium butyrate at 48 h intervals. Doses are expressed in terms of the salts. In an early experiment it was found that of 6 mice pretreated intraventricularly with sodium butyrate (6.8 μg), 5 died after being injected 48 h later with db cyclic AMP (4 or 32 μg). The sodium butyrate did not affect body temperature. No deaths occurred when db cyclic AMP was given before sodium butyrate and this order of injection was therefore used in all the experiments described in this paper.

Db cyclic AMP and sodium butyrate were supplied by the Sigma Chemical Company. Solutions for injection were filtered through Millipore filters (pore size 0.22 μm). The microinjection system and all glassware, filters and saline used were sterile and pyrogen-free as described by Dascombe & Milton (1975).

Differences between the means of experimental data have been evaluated by Student's *t* test ($P >$ or < 0.05) and limits of error are expressed as standard errors.

Results

Effects of dibutyryl cyclic AMP on body temperature and oxygen consumption rate at an ambient temperature of 22°C

Nine mice were each given three successive injections, two of db cyclic AMP (4.0 and 32.0 μg) followed by

Table 1 Effects of dibutyryl cyclic AMP (db cyclic AMP) on the rectal and paw temperatures of mice at an ambient temperature of 22°C ($n = 9$)

Drug	*Dose* (μg)	*Mean temperature (°C ± se) before (0) and after injection* 0 *min*	+12 *min*	+22 *min*	+32 *min*	+42 *min*	+52 *min*
				Rectal temperature			
Na butyrate	6.8	37.8 ± 0.3	37.8 ± 0.3	37.7 ± 0.3	37.6 ± 0.3*	37.5 ± 0.3*	37.6 ± 0.3
db cyclic AMP	4	37.8 ± 0.2	36.3 ± 0.5**†	36.6 ± 0.5*	37.0 ± 0.4*	37.2 ± 0.2*	37.2 ± 0.2*
db cyclic AMP	32	37.8 ± 0.2	35.8 ± 0.5**‡	34.8 ± 0.6**‡	34.0 ± 0.6**‡	33.9 ± 0.7**‡	34.1 ± 0.8**‡
				Paw temperature			
Na butyrate	6.8	29.5 ± 0.2	29.7 ± 0.2	29.6 ± 0.2	29.4 ± 0.2	29.4 ± 0.2	29.5 ± 0.2
db cyclic AMP	4	29.6 ± 0.6	28.4 ± 0.5*‡	28.2 ± 0.3*‡	28.3 ± 0.3*‡	28.4 ± 0.3*‡	28.4 ± 0.4*‡
db cyclic AMP	32	29.4 ± 0.2	29.0 ± 0.2	28.3 ± 0.3**†	27.4 ± 0.3**‡	26.9 ± 0.4**‡	26.8 ± 0.3**‡

P values (paired *t* test) for differences between pre and postinjection values are indicated by * (<0.05) and ** (<0.01). Differences between db cyclic AMP and sodium butyrate treatments at the same times before or after injection are indicated by † (<0.05) and ‡ (<0.01).

Table 2 Effects of dibutyryl cyclic AMP (db cyclic AMP) on the oxygen consumption rates of mice at an ambient temperature of 22°C ($n = 9$)

Drug	*Dose* (μg)	*Mean oxygen consumption rate (ml 25 g^{-1} h^{-1} ± se) in successive 10 min periods before (−) and after (+) injection* −10 to 0	+2 to +12	+12 to +22	+22 to +32	+32 to +42	+42 to +52
Na butyrate	6.8	177 ± 1	182 ± 4	172 ± 1**	169 ± 2*	166 ± 2**	169 ± 1**
db cyclic AMP	4	176 ± 2	126 ± 7**‡	160 ± 2*‡	166 ± 2†	167 ± 1*	165 ± 1*
db cyclic AMP	32	168 ± 2††	112 ± 4**‡	121 ± 2**‡	124 ± 5**‡	125 ± 1**‡	124 ± 2**‡

The data in this table were obtained in the same experiment as those in Table 1. See footnote to Table 1.

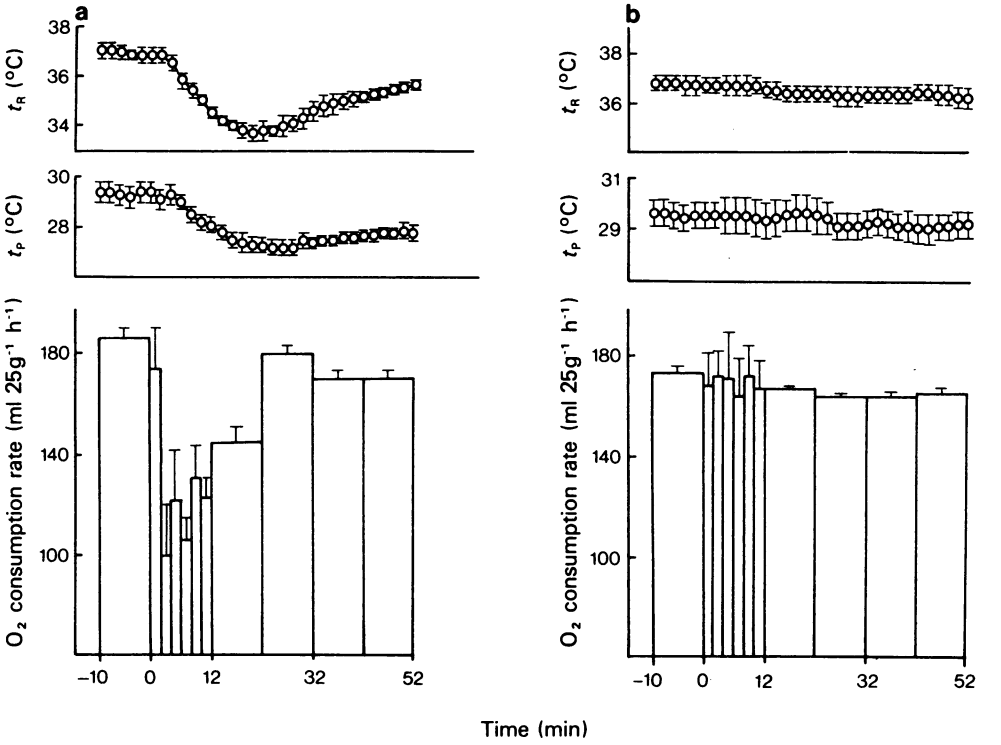

Figure 1(a) The effect of dibutyryl cyclic AMP (16 μg) on the mean rectal temperatures (t_R), paw skin temperatures (t_p) and oxygen consumption rates of a group of 6 mice kept separately at an ambient temperature of 22°C. Injections were made at time zero. Vertical lines show s.e. mean. (b) The effect of sodium *n*-butyrate (6.8 μg) on the mean rectal temperatures (t_R), paw skin temperatures (t_p) and oxygen consumption rates of a group of 6 mice kept separately at an ambient temperature of 22°C. Injections were made at time zero. Vertical lines show s.e. mean.

one of sodium butyrate (6.8 μg). Tables 1 and 2 show that sodium butyrate produced small but significant decreases in both rectal temperature and oxygen consumption rate (V_{O_2}). Db cyclic AMP produced much larger falls and also lowered paw temperature. The size of the effects were related to dose.

In a second experiment six mice were given 16 μg of db cyclic AMP followed by sodium butyrate (6.8 μg). As in the earlier experiment, db cyclic AMP produced significant falls in rectal temperature and V_{O_2} and also in paw temperature (see Figure 1a). Sodium butyrate had no detectable effect (see Figure 1b). The effects of 16 μg db cyclic AMP were similar in size but of shorter duration than those produced by 32 μg in the previous experiment.

With all three doses of db cyclic AMP studied, maximum falls in V_{O_2} occurred before the onset of maximum hypothermia (see Figures 1a and 2 and Tables 1 and 2). Furthermore, in mice given 4 or 16 μg of db cyclic AMP, V_{O_2} was already returning towards preinjection levels at the time of maximum hypothermia. For example, Figure 2 shows that in mice given 4 μg of the drug, hypothermia was first detected 6 min after injection and became maximal 6 min later. The maximum fall in V_{O_2} took place within 2 min of injection and at the time of maximum hypothermia V_{O_2} had already risen significantly above its earlier minimum value (paired *t* test).

It is noteworthy that during the onset of hypothermia induced by db cyclic AMP mice remained inactive and adopted an extended posture. After administration of 16 or 32 μg of the drug there were also intermittent episodes in which the head was shaken from side to side and the forelegs stiffened so that the front part of the torso became raised above the supporting surface. Salivation and lacrymation were also observed frequently after treatment with 32 μg.

Effects of ambient temperature on changes in body temperature and oxygen consumption rate induced by db cyclic AMP (4 μg)

Six mice were each given three successive injections. The first two injections were of db cyclic AMP, one at

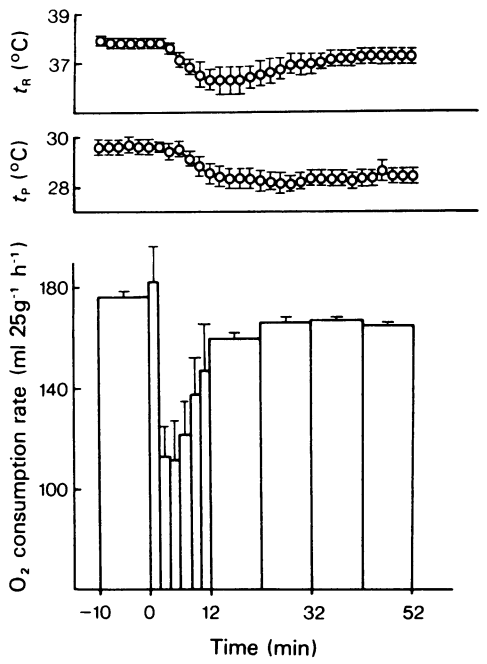

Figure 2 The effect of dibutyryl cyclic AMP (4 μg) on the mean rectal temperatures (t_R), paw skin temperatures (t_p) and oxygen consumption rates of a group of 9 mice kept separately at an ambient temperature of 22°C. Injections were made at time zero. Vertical lines show s.e. mean.

an ambient temperature of 22°C and the other at 32°C. Half the mice received their first injection at 32°C and the other half at 22°C. The final injection was of sodium butyrate (1.7 μg) at 32°C.

Tables 3 and 4 confirm that db cyclic AMP can lower rectal temperature, paw temperature and V_{O_2} at 22°C and show that the drug can also do so at 32°C. At this higher ambient temperature the drug effects were less than at 22°C. For example, between 2 and 12 min after injection, mean falls in V_{O_2} below preinjection values obtained in the 10 min before injection were significantly less ($P < 0.02$) at 32°C (14 ± 8 ml 25 g^{-1}h^{-1}) than at 22°C (59 ± 4 ml 25 g^{-1}h^{-1}). Rectal temperature fell during the first 12 min after injection by 1.0 ± 0.1°C at 32°C and 2.6 ± 0.1°C at 22°C ($P < 0.01$). Sodium butyrate had no effect on body temperature or on V_{O_2} at 32°C.

The above experiment was repeated with six different mice at ambient temperatures of 22° and 36°C instead of 22° and 32°C. Again db cyclic AMP lowered rectal temperature, paw temperature and V_{O_2} at 22°C. However, neither drug nor sodium butyrate had any effect at 36°C (see Tables 5 and 6).

Discussion

Central administration of db cyclic AMP can alter deep body temperature in several species and it is possible therefore that endogenous cyclic AMP may have a role in thermoregulation. Localized application of db cyclic AMP to the preoptic/anterior hypothalamic nuclei produces hyperthermia in the rat (Breckenridge & Lisk, 1969), the rabbit (Laburn, Rosendorff, Willies & Woolf, 1974) and the fowl (Marley & Nistico, 1972) but a fall in body temperature in cats (Dascombe & Milton, 1975). In addition, hypothermia is observed in rabbits before the onset of hyperthermia when db cyclic AMP is injected intracerebroventricularly (i.c.v.) (Duff, Cranston & Luff, 1972; Philip-Dormston & Siegert, 1975). Biphasic

Table 3 Effects of dibutyryl cyclic AMP (db cyclic AMP 4 μg) and sodium butyrate (1.7 μg) on the rectal and paw temperatures of mice at ambient temperatures of 22 and 32°C ($n = 6$)

Drug	t_a (°C)	*Mean temperature (°C ± s.e.) before (0) and after injection* 0 *min*	+12 *min*	+22 *min*	+32 *min*	+42 *min*	+52 *min*
			Rectal temperature				
Na butyrate	32	38.5 ± 0.1	38.4 ± 0.1	38.2 ± 0.2	38.2 ± 0.2	38.3 ± 0.2	38.4 ± 0.1
db cyclic AMP	32	38.3 ± 0.2	37.3 ± 0.1**‡	37.4 ± 0.2**†	37.5 ± 0.2*†	37.5 ± 0.2*†	37.6 ± 0.2**‡
db cyclic AMP	22	37.4 ± 0.2	34.8 ± 0.2**	35.3 ± 0.4**	36.0 ± 0.3**	36.4 ± 0.2*	36.4 ± 0.2*
			Paw temperature				
Na butyrate	32	35.9 ± 0.3	36.1 ± 0.5	36.0 ± 0.5	36.0 ± 0.5	35.9 ± 0.3	36.1 ± 0.4
db cyclic AMP	32	35.6 ± 0.3	34.7 ± 0.2**‡	34.1 ± 0.1**†	34.2 ± 0.1**†	34.3 ± 0.1**‡	34.4 ± 0.1**‡
db cyclic AMP	22	29.2 ± 0.5	28.2 ± 0.5**	27.7 ± 0.4**	27.9 ± 0.3**	28.1 ± 0.2*	28.4 ± 0.2

P values for differences between data obtained at different ambient temperatures (t_a) are not shown. See footnote to Table 1.

changes in body temperature, a fall in temperature succeeded by a rise, elicited by db cyclic AMP injected i.c.v. in rabbits, and also in cats (Varagić & Beleslin, 1973; Clark, Cumby & Davis, 1974) may be a consequence of the nucleotide reaching more than one thermoregulatory site in the brain.

Doggett & Spencer (1971) found db cyclic AMP (25 μg i.c.v.) was without effect on body temperature in the mouse. In contrast the results presented here show that db cyclic AMP lowers body temperature in the restrained mouse. Hypothermia lasted about 1 h and was associated with a fall in oxygen consumption. The falls in oxygen consumption produced by db cyclic AMP always preceded the falls in body temperature in this study, and in many experiments a significant reversal of the effect on oxygen consumption had

Table 4 Effects of dibutyryl cyclic AMP (db cyclic AMP 4 μg) and sodium butyrate (1.7 μg) on the oxygen consumption rates of mice at ambient temperatures of 22 and 32°C ($n = 6$)

Drug	t_a	*Mean oxygen consumption rate (ml 25 g^{-1} h^{-1} ± s.e.) in successive 10 min periods before (−) and after (+) injection*					
	(°C)	−10 to 0	+2 to +12	+12 to +22	+22 to +32	+32 to +42	+42 to +52
Na butyrate	32	72 ± 2	81 ± 2	66 ± 3	73 ± 1	72 ± 6	72 ± 1
db cyclic AMP	32	77 ± 2	63 ± 2*‡	66 ± 2**	69 ± 3	77 ± 0	78 ± 0†
db cyclic AMP	22	144 ± 3	85 ± 9**	127 ± 9	139 ± 2	139 ± 2	137 ± 1

The data in this table were obtained in the same experiment as those in Table 3. *P* values for differences between data obtained at different ambient temperatures (t_a) are not shown. See footnote to Table 1.

Table 5 Effects of dibutyryl cyclic AMP (db cyclic AMP 4 μg) and sodium butyrate (1.7 μg) on the rectal and paw temperatures of mice at ambient temperatures of 22 and 36°C ($n = 6$)

Drug	t_a	*Mean temperature (°C ± s.e.) before (0) and after injection*					
	(°C)	0 *min*	+12 *min*	+22 *min*	+ 32 *min*	+42 *min*	+52 *min*
				Rectal temperature			
Na butyrate	36	39.9 ± 0.3	39.7 ± 0.5	39.5 ± 0.5	39.4 ± 0.4*	39.3 ± 0.3*	39.2 ± 0.3**
db cyclic AMP	36	40.1 ± 0.3	39.7 ± 0.1	39.8 ± 0.3	39.7 ± 0.4	39.5 ± 0.2	39.4 ± 0.1
db cyclic AMP	22	38.0 ± 0.2	34.8 ± 0.5**	34.6 ± 0.7**	35.5 ± 0.6**	36.2 ± 0.5**	36.4 ± 0.4**
				Paw temperature			
Na butyrate	36	35.4 ± 2.0	37.0 ± 1.7	36.9 ± 1.7	36.8 ± 1.6	36.8 ± 1.6	36.8 ± 1.5
db cyclic AMP	36	38.6 ± 0.3	38.4 ± 0.2	38.5 ± 0.2	38.5 ± 0.3	38.3 ± 0.2	38.2 ± 0.1
db cyclic AMP	22	29.0 ± 0.3	27.5 ± 0.4*	26.8 ± 0.5*	27.0 ± 0.6**	27.2 ± 0.6*	27.4 ± 0.7*

See footnotes to Tables 1 and 3.

Table 6 Effects of dibutyryl cyclic AMP (db cyclic AMP 4 μg) and sodium butyrate (1.7 μg) on the oxygen consumption rates of mice at ambient temperatures of 22 and 36°C ($n = 6$)

Drug	t_a	*Mean oxygen consumption rate (ml 25 g^{-1} h^{-1} ± s.e.) in successive 10 min periods before (−) and after (+) injection*					
	(°C)	−10 to 0	+2 to +12	+12 to +22	+22 to +32	+32 to +42	+42 to +52
Na butyrate	36	69 ± 3	80 ± 3*	78 ± 3*	86 ± 3*	87 ± 1**	84 ± 3*
db cyclic AMP	36	82 ± 2†	86 ± 6	86 ± 4	82 ± 2	88 ± 1	85 ± 3
db cyclic AMP	22	157 ± 3	89 ± 4**	115 ± 7**	147 ± 4*	161 ± 1	161 ± 2

See footnotes to Tables 1 and 4.

already occurred at the time maximum hypothermia was observed. These results indicate not only that the hypothermic effect of centrally applied db cyclic AMP is associated with a fall in heat production in the restrained mouse but also that there is a cause and effect relationship between these events, the hypothermia resulting from an effect of the nucleotide on heat production. However, the observation that rectal temperature changes lagged behind changes in oxygen consumption may be attributable to differences in the reaction times of the methods used to measure these changes and caution must be exercised in interpreting the temporal sequence of these events. Restrained mice show increases in oxygen consumption and therefore presumably in heat production in response to decreases in ambient temperature below 34°C (Pertwee & Tavendale, 1977). Both hypothermia and decreased oxygen consumption induced by db cyclic AMP increased both with dose of drug and with reduction in ambient temperature. At an ambient temperature of 36°C, db cyclic AMP had no detectable effect on either rectal temperature or oxygen consumption. It appears therefore that db cyclic AMP may inhibit the central processes associated with increased heat production in response to cold.

Doses of db cyclic AMP which lowered rectal temperature in the mouse also produced falls not rises in paw temperature. These falls occurred at the same time as the falls in rectal temperature and are therefore probably passive effects following the reduction in core temperature rather than active changes caused by vasoconstriction. It is unlikely therefore that decreases in peripheral vasomotor tone and consequent heat loss contributed significantly towards the hypothermia induced by the drug. Even so, hypothermia did seem to depend to some extent on increased heat loss as well as on decreased heat production. After drug administration, animals adopted an extended posture exposing as much as possible of their body surface to the environment. Piloerection was absent in mice after injection of db cyclic AMP. Non-thermoregulatory effects of db cyclic AMP injected into the cerebroventricular system included lacrymation and lateral head movements. The factors responsible for the deaths of 5 mice from a group of 6 animals receiving db cyclic AMP 48 h after injection of sodium *n*-butyrate in an early experiment in this study have not yet been elucidated.

The injection site used in these experiments was in the third cerebral ventricle close to the hypothalamus and it is possible that db cyclic AMP lowered heat production by an action on hypothalamic thermoregulatory centres. However, it should be noted, that other sites of action cannot be excluded. After injection the nucleotide would probably have diffused to many parts of the central nervous system. For example, it is likely that the head movements produced by the highest dose of drug (32 μg) were caused by an effect on extrahypothalamic sites.

It is concluded that db cyclic AMP injected i.c.v. in the mouse produces a fall in rectal temperature which is effected at least in part by decreased heat production, an absence of piloerection and the adoption of an extended posture. These effects constitute pharmacological responses of the mouse to central injection of an exogenous derivative of cyclic AMP, db cyclic AMP which is often presumed to mimic the actions of endogenous nucleotide. However, it should be noted that db cyclic AMP or its metabolites may not necessarily act by altering the activity of intracellular cyclic-AMP-dependent kinases and may even act extracellularly. Hence the possible physiological significance of hypothermia induced by db cyclic AMP requires further study.

We thank the Medical Research Council for support (M.J.D.). I.N-A. is the recipient of a Ghana Government Scholarship.

References

BRECKENRIDGE, B. McL. & LISK, R.D. (1969). Cyclic adenylate and hypothalamic regulatory functions. *Proc. Soc. exp. Biol. Med.*, **131**, 934–935.

CLARK, W. G., CUMBY, H.R. & DAVIS, H.E. (1974). The hyperthermic effect of intracerebroventricular cholera enterotoxin in the unanaesthetized cat. *J. Physiol.*, **240**, 493–504.

DASCOMBE, M.J. & MILTON, A.S. (1975). The effects of cyclic adenosine 3',5'-monophosphate and other adenine nucleotides on body temperature. *J. Physiol.*, **250**, 143–160.

DOGGETT, N.S. & SPENCER, P.S.J. (1971). Pharmacological properties of centrally administered ouabain and their modification by other drugs. *Br. J. Pharmac.*, **42**, 242–253.

DRUMMOND, G.I. & MA, Y. (1975). Metabolism and functions of cyclic AMP in nerve. In *Progress in Neurobiology*, Vol. 2. ed. Kerkut, G.A. & Phyllis, J.W. pp. 119–179, Oxford: Pergamon Press.

DUFF, G.W., CRANSTON, W.I. & LUFF, R.H. (1972). Cyclic 3',5'-adenosine monophosphate in central control of body temperature. *Proc. 5th International Congress of Pharmacology* (Abstract) No. 360, p. 60.

HENION, W. F., SUTHERLAND, E.W. & POSTERNAK, T. (1967). Effects of derivatives of adenosine 3',5'-phosphate on liver slices and intact animals. *Biochim. biophys. Acta*, **148**, 106–113.

KEBABIAN, J.W. (1977). Biochemical regulation and physiological significance of cyclic nucleotides in the nervous system. In *Adv. Cyclic Nucleotide Res.* ed. Greengard,

P. & Robison, G.A. pp. 421–508 New York: Raven Press.

LABURN, H., ROSENDORFF, C., WILLIES, G. & WOOLF, C. (1974). A role for noradrenaline and cyclic AMP in prostaglandin E_1 fever. *J. Physiol.*, **240**, 49–50P.

MARLEY, E. & NISTICO, G. (1972). Effects of catecholamines and adenosine derivatives given into the brain of fowls. *Br. J. Pharmac.*, **46**, 619–636.

NATHANSON, J.A. (1977). Cyclic nucleotides and nervous system function. *Physiol. Rev.*, **57**, 157–256.

NYEMITEI-ADDO, I., PERTWEE, R.G. & TAVENDALE, R. (1980). Stereotaxic implantation of cannulae for subsequent drug administration into the third ventricles of conscious mice. *Br. J. Pharmac.*, **68**, 189P.

PERTWEE, R.G. (1970). The effects of anaesthetic gases at high pressure on thermoregulation. *D. Phil. Thesis, Oxford.*

PERTWEE, R.G. (1974). Tolerance to the effects of Δ^1-tetrahydrocannabinol on corticosterone concentrations in mouse plasma produced by repeated administration of cannabis extract or Δ^1-tetrahydrocannabinol. *Br. J. Pharmac.*, **51**, 391–397.

PERTWEE, R.G. & TAVENDALE, R. (1977). Effects of Δ^9-tetrahydrocannabinol on the rates of oxygen consumption of mice. *Br. J. Pharmac.*, **60**, 559–568.

PHILIPP-DORMSTON, W.K. & SIEGERT, R. (1975). Fever produced in rabbits by N^6,$O^{2'}$-dibutyryl adenosine 3',5'-cyclic monophosphate. *Experientia*, **31**, 471–472.

POSTERNAK, T., SUTHERLAND, E.W. & HENION, W.F. (1962). Derivatives of cyclic 3',5'-adenosine monophosphate. *Biochim. biophys. Acta*, **65**, 558–560.

VARAGIĆ, V.M. & BELESLIN, D.B. (1973). The effects of N-2-O-dibutyryl-adenosine-3',5'-monophosphate, adenosine triphosphate and butyrate on the body temperature of conscious cats. *Brain Res.*, **57**, 252–254.

(Received October 3, 1979.
Revised January 3, 1980.)