Biochemical Pharmacology, Vol. 21, pp. 2615–2626. Pergamon Press, 1972. Printed in Great Britain.

EFFECT OF CYCLIC 3′,5′-MONOPHOSPHATE ON EDEMA AND GRANULOMA INDUCED BY CARRAGEENIN

ATSUSHI ICHIKAWA, MASAKO NAGASAKI, KOHEI UMEZU, HIDEYA HAYASHI and KENKICHI TOMITA

Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan

(*Received* 2 *January* 1972; *accepted* 10 *April* 1972)

Abstract—The anti-inflammatory activity of cyclic adenosine 3′,5′-monophosphate (cAMP) alone or in combination with theophylline was examined in acute and chronic types of experimental inflammations in rats. cAMP suppressed carrageenin-induced edema by intraperitoneal, subcutaneous and oral administrations. In addition, cAMP inhibited the formation of carrageenin-induced granuloma. Systemic applications of cAMP also suppressed the leakage of circulating dye in rat skins induced by moderate thermal injury and by histamine and inorganic pyrophosphate, but they suppressed less effectively the leakage induced by exogenous bradykinin. Theophylline always augmented the suppressive effects of cAMP in these inflammations. A single intraperitoneal injection of cAMP alone or in combination with theophylline induced a slight, transient hyperglycemia and did not significantly alter the plasma level of corticoids. It seems unlikely that the anti-inflammatory action of cAMP is mediated solely by hyperglycemia or by increased production of corticosteroids.

IN A previous paper,[1] we reported that subcutaneous injection of inorganic pyrophosphate (PP_i) or ATP produced inflammatory lesions on rat skins. This PP_i- or ATP-induced inflammation was characterized by immediate and monophasic increases in vascular permeability and cutaneous histamine content. We also reported that simultaneous subcutaneous injection of cyclic adenosine 3′,5′-monophosphate (cAMP) effectively inhibited the increase in vascular permeability induced by PP_i, ATP or exogenous histamine, but it inhibited less effectively the increase induced by bradykinin. In addition, cAMP was also effective in inhibiting the release of histamine from isolated mast cells of rats, but not from isolated leukocytes.

Since Bertelli *et al.*[2] reported the suppressive effects of cAMP on edemas of the foot pads of rats induced by some phlogistins, we have further investigated the effects of cAMP on several experimental inflammations of the acute and chronic type. Besides dye leakage in rat skins induced by various phlogistins including thermal injury, we employed carrageenin-induced edema on rat foot pads as a model of acute inflammation, because it has been known that carrageenin gives more consistent results than other inflammatory agents such as formalin, dextran and egg white,[3] and also that the effectiveness of drugs in carrageenin-induced edema often correlates with their effectiveness in rheumatic diseases.[4] As a model of a more chronic type of inflammation[4] and of a healing wound,[5] we used carrageenin-induced granuloma on the dorsum of rats.

In this report, effects of cAMP administered by various routes to rats on these experimental inflammations are described and compared with those of some other anti-inflammatory agents.

METHODS AND MATERIALS

Carrageenin-induced edema in hind paws of rat. The method of Winter *et al.*[3] as modified by Niemegeers *et al.*[6] was used. Young adult male rats of the Wistar strain weighing 150 ± 10 g were maintained with water and laboratory Chow (CE-2 pellet, Nihon Clea Co., Tokyo, Japan) *ad lib.* Adrenalectomy, when required, was performed 4 days before use and the operated animals were given 1% NaCl to drink. Drugs to be tested were suspended in water or in a 1% carboxymethyl cellulose solution at pH 6–7 and were given through a stomach tube (1 ml/100 g of body wt), followed by distilled water (4 ml/rat). Control animals received only distilled water (5 ml). A 1% suspension of carrageenin (Seakem 202, kindly supplied by Dr. S. Tsurufuji, Faculty of Pharmaceutical Sciences, University of Tokyo, Japan) in 0·9% saline was prepared fresh daily about 1 hr prior to its injection (0·05 ml) into the plantar side of the right-hind paw 1 hr after the oral administration of drugs. For comparison, drugs were also administered subcutaneously or intraperitoneally (0·5 ml/100 g body wt) 30 min before the injection of carrageenin. In most cases, the degree of swelling in the hind paw was measured 3 hr after carrageenin treatment. As a control, the same carrageenin solution was injected into the left hind paw of the rat immediately before sacrifice. After decapitation, both feet were severed at the ankle joint and weighed. Instead of measuring foot volume with an electric antiphlogometer as did Van Arman *et al.*,[7] the increase in weight of the hind paw over its control was used to express the degree of swelling, and the per cent of inhibition of edema by drug treatment over the vehicle treatment was calculated.

Carrageenin-induced granuloma. Granuloma pouch was induced in male rats of the Donryu strain weighing 100 ± 10 g by the method of Fukuhara and Tsurufuji.[8] A sterilized carrageenin solution in 0·9% NaCl (2%, w/v; 4 ml) was injected into the air sac pre-formed by injecting air subcutaneously 1 day before on the dorsum of the animal. Drugs were tested for their inhibitory effects on the formation of granuloma and for their reducing effects on the preformed granuloma. For the former effect, drugs were injected once daily into the granuloma pouch or intraperitoneally for 5 days beginning on the day of carrageenin injection. On the sixth day, the animals were decapitated, and the entire granuloma pouch was carefully removed. The pouch was incised to harvest "pouch fluid", then its volume and the wet weight of remaining "pouch wall" were measured. For the latter effect, drugs were similarly administered once daily for 5 days beginning on the sixth day after the carrageenin injection. The volume of pouch fluid and the wet wt of the pouch wall were measured on day 11, with samples on day 6 serving as controls.

Thermal injury test. Male rats of the Wistar strain weighing 150 ± 10 g were used. One hr before the infliction of burns, animals received an intraperitoneal injection of drugs dissolved or suspended in 0·9% NaCl (0·5 ml/100 g body wt) or of the vehicle only as the control, followed 30 min later by an injection of 1% Evans blue in 0·9% NaCl through the tail vein (0·2 ml/100 g) under anesthesia with sodium pentobarbital (50 mg/kg, intraperitoneal). Thermal injury was produced by a modified apparatus as described by Spector and Willoughby.[9] Six cupper cylinders (6 mm in diameter, one end closed, surface-polished and protruding 5 mm through an asbestos plate) were fixed 1 cm apart in a 2 × 3 cm area on the top surface of a cupper box. The temperature was maintained at 58°or 60° by circulating water through the box from a constant temperature bath. Rats, anesthetized with ether and with their ventral skin

closely clipped, were placed over the six cupper cylinders for 27 sec at 58° for a mild injury or for 45 sec at 60° for a severe burn. Animals were killed immediately after the turn by decapitation and the skin of the burnt area (1 × 1 cm) was excised. The cutaneous dye was extracted and assayed by the method of Beach and Steinetz,[10] as described in a previous paper.[1]

Vascular permeability test. Changes in vascular permeability were measured in a way similar to that described previously.[1] Rats were intraperitoneally injected with cAMP or theophylline (each 100 mg/kg body wt), or both; then 30 min later they were injected with 0·05 ml of 1% Evans blue in 0·9% NaCl through the tail vein under anesthesia with sodium pentabarbital (50 mg/kg, i.p.). Another 10 min later, various inflammatory agents (histamine, bradykinin and PP_1) dissolved in 0·05 ml of 0·9% NaCl (pH 7·4) were subcutaneously injected into selected sites of the closely clipped ventral skin. Thirty min later, the amount of Evans blue at the injected areas (1 × 1 cm) was assayed as described previously.[1]

Other assays. Unconjugated cortisol and corticosterone in plasma were determined by the fluorometric method of Nielsen and Asfeldt.[11] Blood glucose was estimated by the *o*-toluidine method,[12] after deproteinization of a blood sample from the carotid artery with trichloroacetic acid.

Glycogen in liver was precipitated from alkaline-digested liver samples with alcohol[13] and estimated as glucose by the *o*-toluidine method.[12]

cAMP was obtained from Seishin Parmaceutical Co., Tokyo, Japan. Other chemicals of reagent grade and drugs were obtained commercially.

RESULTS

Effect of cAMP on carrageenin-induced edema. Maximum swelling measured by the weight increase of the hind paws occurred about 3 hr after the injection of carrageenin and was in a good agreement with results reported by other investigators who measured changes in the volume of hind paws.[3,6] In a typical experiment, increases in swelling weight, after the injection of 0·05 ml of 1% carrageenin solution, were 0·38 ± 0·09 g at 1 hr, 0·51 ± 0·07 at 2 hr, 0·88 ± 0·10 at 3 hr and 0·60 ± 0·13 at 5 hr. Therefore, in most of the subsequent studies, effects of various drugs were estimated 3 hr after the injection of carrageenin.

As shown in Table 1, cAMP administered intraperitoneally to rats was fairly effective in suppressing the formation of carrageenin-induced edema. Its half-effective dose (ED_{50}) calculated from dose–response curves was 18 mg/kg of body wt, one-sixth as effective as indomethacin, but twice as potent as phenylbutazone and about 10 times as potent as hydrocortisone.

Figure 1 shows that cAMP was also anti-edematic when administered orally or subcutaneously, and the suppressive effects were proportional to the logarithmic doses of cAMP.

As shown in Table 2, this anti-edematic potency of cAMP was augmented by simultaneous injection of theophylline (50 mg/kg), which itself was only slightly anti-edematic (Table 1). The suppressive effect of cAMP and the augmentative effect of theophylline were also observed with adrenalectomized rats. However, in these animals, the size of carrageenin-induced edema was about 1·5 times larger than that in normal rats, and the suppressive effect of cAMP was slightly reduced.

TABLE 1. EFFECTS OF cAMP AND VARIOUS OTHER COMPOUNDS ON CARRAGEENIN-INDUCED EDEMA*

Compound	Doses, i.p. (mg/kg)	No. of rats	Inhibition (%)
cAMP	1	9	10 ± 2·5
	10	8	35 ± 3·0
	50	6	70 ± 4·1
Theophylline	10	6	4·6 ± 3·2
	50	6	18 ± 4·0
	100	6	27 ± 3·1
cAMP plus theophylline (50 mg/kg)	1	6	17 ± 2·9
	10	6	45 ± 5·8
	25	6	69 ± 5·1
	50	6	84 ± 4·8
Indomethacin	1	6	20 ± 2·9
	5	6	57 ± 4·8
	10	6	80 ± 5·7
Mefenamic acid	20	6	39 ± 2·2
	50	6	71 ± 3·1
Flufenamic acid	10	6	29 ± 4·0
	50	6	64 ± 4·5
Phenylbutazone	10	6	18 ± 3·2
	40	6	43 ± 3·1
Hydrocortisone	10	6	13 ± 1·2
	50	6	25 ± 1·2
Na salicylate	50	5	12 ± 1·4
	100	4	42 ± 3·1

* Rats were injected intraperitoneally with each compound 30 min before the injection of carrageenin solution (1%) into the plantar side of the right hind paw. Three hr later, the same carrageenin solution was injected into the plantar side of the left hind paw immediately before sacrifice of the animals. Control rats were injected with 0·9% saline instead of solutions of drugs. The degree of swelling expressed as the weight increase of the hind paw (right minus left) was measured 3 hr after the carrageenin injection and the per cent of inhibition over the control was calculated.

Effect of cAMP on thermal injury. Intraperitoneal administration of cAMP (100 mg/kg of body wt) prior to the infliction of burn significantly suppressed the increase in vascular permeability due to mild thermal injury (58° for 27 sec) but not the increase due to severe injury (60° for 45 sec). Simultaneous administration of theophylline augmented the suppressive effect of cAMP significantly in mild injury and slightly in severe injury (Table 3).

Effect of cAMP on changes in vascular permeability induced by histamine, bradykinin and PP_i. In a previous paper,[1] we reported that simultaneous subcutaneous injection of cAMP together with PP_i, histamine and bradykinin effectively inhibited the increase in vascular permeability induced by these phlogistic agents. Similar suppressive effects of cAMP were observed with its systemic application. Table 4 shows that prior intraperitoneal injection of cAMP (100 mg/kg) effectively suppressed (30–40 per cent the increase in vascular permeability induced by exogenous histamine, bradykinin and

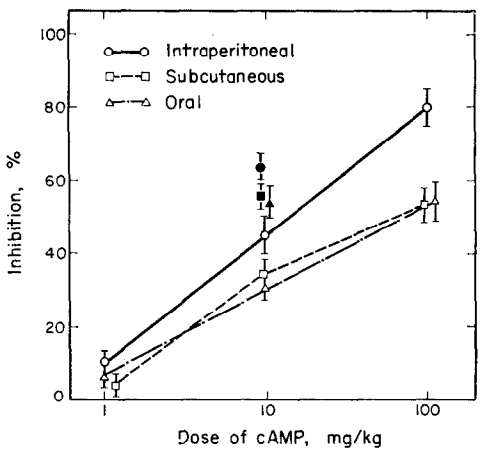

FIG. 1. Suppressive effect of cAMP by intraperitoneal, subcutaneous or oral administration on carrageenin-induced edema. Rats were given various doses of cAMP intraperitoneally (○——○), subcutaneously (□——□) (both at 0·5 ml/100 g of body weight) or orally (△-·-·-△) (in 1 ml/100 g). Edema was introduced by injecting a 1% carrageenin solution (0·05 ml) into the plantar side of the hind paws 30 min after the injection of cAMP or 1 hr after its oral administration. The effect of theophylline (50 mg/kg) (●, ■, ▲) was examined by administering it mixed with 10 mg/kg of cAMP. The degree of swelling was measured 3 hr after the carrageenin injection, as described in the text and in Table 1. Each point represents the mean of seven to nine rats ± S.E.

PP_i. Doses of cAMP below 100 mg/kg were not effective. As with the above, theophylline (100 mg/kg, i.p.) further elevated the suppressive effect of cAMP to 50–60 per cent inhibition. Under the experimental conditions used, cAMP was more effective in suppressing the inflammatory effect induced by PP_i than that by histamine or bradykinin, but its overall inhibitory effects by intraperitoneal injection were weaker than those observed by simultaneous subcutaneous injection.

TABLE 2. EFFECT OF cAMP WITH OR WITHOUT THEOPHYLLINE ON CARRAGEENIN-INDUCED EDEMA IN THE HIND PAWS OF NORMAL AND ADRENALECTOMIZED RATS*

cAMP, theophylline (i.p. doses)	No. of rats	Degree of swelling (g ± S.E.)	Inhibition (%)
Normal rats			
None	7	0·92 ± 0·10	
cAMP (25 mg/kg)	7	0·42 ± 0·11	53
cAMP (25 mg/kg) plus Theophylline (50 mg/kg)	6	0·30 ± 0·08	67
Adrenalectomized rats			
None	6	1·28 ± 0·19	
cAMP (25 mg/kg)	6	0·78 ± 0·08	39
cAMP (25 mg/kg) plus Theophylline (50 mg/kg)	6	0·60 ± 0·05	53

* Experimental procedures are described in the text.

TABLE 3. EFFECT OF cAMP AND THEOPHYLLINE ON LEAKAGE OF CIRCULATING EVANS BLUE INTO THE SKIN OF RATS AFTER THERMAL INJURY*

Treatment (doses, i.p.)	No. of rats	Evans blue/(1 cm)²	
		58° for 27 sec†	60° for 45 sec†
0·9% Saline	6	20·5 ± 2·0	32·8 ± 3·0
cAMP (10 mg/kg)	3	18·0 ± 1·0 (12)	30·1 ± 2·0 (8)
(50 mg/kg)	3	15·2 ± 3·4 (26)	26·9 ± 3·1 (18)
(100 mg/kg)	5	9·6 ± 1·2 (53)‡	24·0 ± 2·3 (27)
Theophylline (50 mg/kg)	6	14·5 ± 1·4 (30)§	29·8 ± 2·9 (9)
cAMP (100 mg/kg) plus theophylline (50 mg/kg)	6	5·9 ± 1·0 (71)‡	21·9 ± 3·2 (33)§

* One hr before inflicting the burns, rats were intraperitoneally injected with drugs in 0·9% NaCl (0·5 ml/100 g of body weight) or with the vehicle only as control. Thirty min later they were injected with 1% Evans blue in 0·9% NaCl (0·3 ml/100 g) through the tail vein. Thermal injury was produced by a modified apparatus of the Spector and Willoughby[9] type as described in the text. Evans blue in the burnt area (1 × 1 cm) was extracted and assayed by the method of Beach and Steinetz.[10]

† The per cent inhibition is given in parentheses.

‡ $P < 0.02$.

§ $P < 0.05$.

Effect of cAMP on carrageenin-induced granuloma. Granuloma induced by carrageenin reached their maximum size on the sixth day after the injection of a 1% carrageenin solution (4 ml) into the air sac on the dorsum. In control rats, the volume of pouch fluid on the sixth day was about 7–8 ml and the wet wt of pouch wall

TABLE 4. EFFECTS OF cAMP AND/OR THEOPHYLLINE ON LEAKAGE OF CIRCULATING EVANS BLUE INTO THE SKIN OF RATS INDUCED BY HISTAMINE, BRADYKININ AND PP_i*

Drugs (doses, i.p.)	Evans blue μg/(1 cm)²		
	Histamine†	Bradykinin†	PP_i†
0·9% Saline	38·0 ± 3·2	36·6 ± 3·5	27·4 ± 1·9
cAMP (100 mg/kg)	26·4 ± 2·5 (30)‡	30·0 ± 2·6 (18)	17·8 ± 1·3 (35)‡
Theophylline (100 mg/kg)	30·0 ± 1·8 (21)	29·0 ± 2·8 (21)	22·0 ± 1·5 (20)
cAMP + theophylline (100 mg/kg each)	21·0 ± 1·9 (45)§	25·6 ± 1·5 (30)‡	14·0 ± 1·3 (48)§

* Rats were intraperitoneally injected with cAMP (100 mg/kg of body weight) and/or theophylline (100 mg/kg). Thirty min later they were injected with 1% Evans blue solution in 0·9% NaCl (0·3 ml/kg) through the tail vein. Another 10 min later, histamine (0·5 μmole), bradykinin (1 μg) or PP_i (0·5 μmole) was subcutaneously injected at selected sites on the closely clipped ventral skin. Thirty min later, the amount of Evans blue at the injected areas (1 × 1 cm) was assayed as described previously.[1] All data represent the means of values from six rats ± S.E.

† The per cent inhibition is given in parentheses.

‡ $P < 0.05$.

§ $P < 0.02$.

was 6–7 g. cAMP strongly inhibited the formation of granuloma. Table 5 shows that, in rats receiving 0·5–10·0 mg cAMP daily in the carrageenin-induced pouches, both the wet weight of pouch wall and the volume of pouch fluid were significantly reduced as the dose of cAMP was increased.

TABLE 5. EFFECTS OF cAMP AND/OR THEOPHYLLINE ON THE FORMATION OF CARRAGEENIN-INDUCED GRANULOMA*

Drugs (daily doses)	No. of rats	Body wt increase (g/day)	Pouch fluid† (ml)	Pouch wall wet wt† (g)
(A) Intrapouch administration				
0·9% Saline	14	3·0 ± 0·5	8·2 ± 0·3	7·32 ± 0·56
cAMP (0·5 mg)	9	2·6 ± 0·4	6·9 ± 0·5 (16)	5·83 ± 0·30 (20)
(1·0 mg)	12	2·0 ± 0·2	3·8 ± 0·4 (54)‡	4·08 ± 0·32 (44)‡
(5·0 mg)	10	2·1 ± 0·2	2·4 ± 0·2 (71)§	3·59 ± 0·61 (51)§
(10·0 mg)	15	1·6 ± 0·3	1·7 ± 0·2 (79)§	3·17 ± 0·09 (57)§
Theophylline (5 mg)	8	1·6 ± 0·3	6·9 ± 0·4 (16)	6·10 ± 0·52 (17)
(10 mg)	8	1·5 ± 0·2	5·8 ± 0·5 (29)	5·38 ± 0·49 (26)
cAMP (1·0 mg)				
+ theophylline (5 mg)	8	1·2 ± 0·4	0·5 ± 0·12 (94)§	1·75 ± 0·20 (76)§
+ theophylline (10 mg)	8	0·8 ± 0·3	Negligible (100)§	0·73 ± 0·21 (90)§
cAMP (10·0 mg)				
+ theophylline (5 mg)	8	1·1 ± 0·1	Negligible (100)§	Negligible (100)§
+ theophylline (10 mg)	8	0·4 ± 0·3	Negligible (100)§	Negligible (100)§
(B) Intraperitoneal administration				
0·9% Saline	6	3·8 ± 0·6	7·9 ± 0·8	7·30 ± 0·42
cAMP (10 mg/kg)	6	2·0 ± 0·4	7·0 ± 0·4 (11)	6·60 ± 0·29 (9)
(50 mg/kg)	5	1·3 ± 0·3	5·2 ± 0·6 (34)‡	5·40 ± 0·33 (26)‡
(100 mg/kg)	6	0·5 ± 0·1	4·1 ± 0·4 (48)‡	4·37 ± 0·29 (40)‡
Theophylline (50 mg/kg)	5	0·8 ± 0·2	7·0 ± 0·8 (11)‡	6·69 ± 0·47 (8)
cAMP (100 mg/kg)				
+ theophylline (100 mg/kg)	7	0·3 ± 0·3	3·0 ± 0·3 (62)§	3·12 ± 0·26 (57)§

* Granuloma pouch was induced in male rats of the Donryu strain by injecting a sterilized carrageenin solution in 0·9% NaCl into the air sac pre-formed 1 day before on the dorsum of the animals.[8] Drugs were injected once daily into the granuloma pouch (A) or intraperitoneally (B) for 5 days beginning on the day of carrageenin injection. On day 6, the animals were decapitated, then the entire granuloma pouch was removed. The volume of pouch fluid and the weight of the pouch wall were measured.

† The per cent inhibition is given in parentheses.

‡ $P < 0.05$.

§ $P < 0.02$.

Again this effect of cAMP was augmented by theophylline, which itself was slightly inhibitory. The increase in body weight was not significantly affected in groups receiving lower doses of cAMP, but was suppressed in groups receiving higher doses of cAMP, theophylline, or both. Table 5 also shows that intraperitoneal injection of cAMP, though it required much higher doses, was also effective in suppressing the formation of granuloma and the accumulation of pouch fluid. However, the retardation of body weight increase was more apparent with the intraperitoneal injection of cAMP than with the intra-pouch injection of this nucleotide.

Dose–response curves showed that the inhibitory effect of 10 mg cAMP on the

formation of pouch wall was nearly equal to effects obtained with 1·0 mg indomethacin, 35 mg phenylbutazone, 25 mg flufenamic acid or 15 mg hydrocortisone. In suppressing the accumulation of pouch fluid, 10 mg cAMP was as potent as 2·0 mg indomethacin, 25 mg phenylbutazone, 9 mg mephenamic acid and 10 mg flufenamic acid.

In contrast, the effect of cAMP on the pre-formed granuloma was markedly different. Table 6 shows that with injection of 1 or 10 mg cAMP, or 10 mg theophylline into pre-formed granuloma for 5 days, beginning on the sixth day, both the wet wt of pouch wall and the volume of pouch fluid were increased or were not significantly decreased as compared with the controls. However, a higher dose of cAMP (50 mg/pouch) or the combination of cAMP with theophylline was effective in reducing granuloma. Similar results were obtained with intraperitoneal injection of cAMP or theophylline, or both, as shown in experiment B of Table 6.

TABLE 6. EFFECTS OF cAMP AND/OR THEOPHYLLINE ON PREFORMED CARRAGEENIN GRANULOMA IN RATS*

Drugs (daily doses)	No. of rats	Body wt increase (g/day)	Pouch fluid† (ml)	Pouch wall wet wt† (g)
(A) Intrapouch administration				
Control (carrageenin-injected)				
(5 days)	6	2·9 ± 0·3	6·0 ± 3·0	5·4 ± 0·3
(10 days)	6	3·0 ± 0·2	8·5 ± 1·9 (100)	4·8 ± 0·3 (100)
cAMP (1 mg/pouch)	6	3·2 ± 0·2	11·6 ± 2·4 (137)‡	5·6 ± 0·3 (117)
(10 mg/pouch)	6	3·8 ± 0·3	8·9 ± 3·0 (105)	4·0 ± 0·3 (83)
(50 mg/pouch)	6	2·2 ± 0·2	4·8 ± 1·9 (57)§	3·8 ± 0·2 (79)‡
Theophylline (10 mg/pouch)	6	3·0 ± 0·1	7·1 ± 2·3 (84)	4·3 ± 0·2 (90)
+ cAMP (1 mg/pouch)	6	2·6 ± 0·3	4·9 ± 3·1 (58)‡	4·0 ± 0·3 (83)
+ cAMP (10 mg/pouch)	6	2·4 ± 0·2	3·1 ± 1·6 (56)§	3·2 ± 0·3 (67)‡
(B) Intraperitoneal administration				
Control (carrageenin-injected)				
(5 days)	6	2·8 ± 0·1	5·2 ± 1·9	5·7 ± 0·4
(10 days)	5	3·5 ± 0·2	10·6 ± 3·8 (100)	4·8 ± 0·2 (100)
cAMP (10 mg/kg)	6	2·8 ± 0·2	14·6 ± 3·0 (138)‡	6·2 ± 0·2 (129)
cAMP (100 mg/kg)	6	4·5 ± 0·4	6·2 ± 1·9 (59)‡	3·9 ± 0·4 (81)‡
Theophylline (50 mg/kg)	5	2·6 ± 0·2	8·3 ± 1·5 (78)	4·0 ± 0·3 (83)
+ cAMP (10 mg/kg)	7	3·0 ± 0·3	4·4 ± 2·1 (42)§	3·6 ± 0·8 (75)‡
+ cAMP (100 mg/kg)	7	3·8 ± 0·2	2·5 ± 2·3 (24)§	4·2 ± 0·2 (87)‡

* Granuloma was induced in rats by carrageenin as described in Table 5 and the text. Drugs were administered once daily for 5 days beginning on day 6 after the carrageenin injection. All values represent the mean ± S.E. P values calculated by Student's *t*-test denote the significant difference from the 10-day control.

† The per cent of the 10-day control is given in parentheses.

‡ $P < 0.05$.

§ $P < 0.02$.

Effect of cAMP on blood glucose and corticosteroid secretion. The suppressive effect of cAMP on these experimental inflammations might be due to hyperglycemia or to elevated secretion of the corticosteroids induced by this nucleotide. Figure 2 shows that intraperitoneal injection of cAMP (100 mg/kg) caused a transient hyperglycemia in

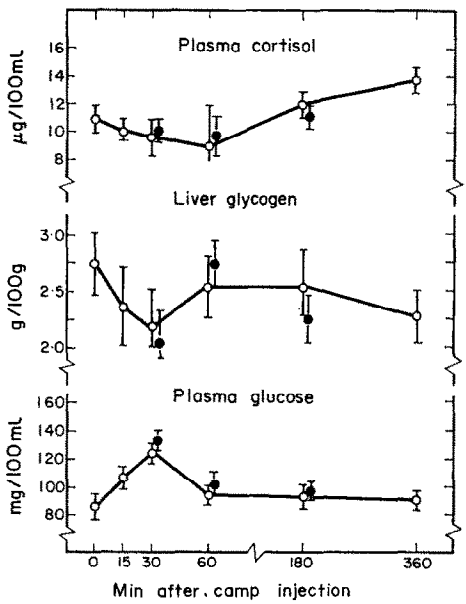

FIG. 2. Effects of cAMP and theophylline on glucose and corticosteroids in the plasma, and on glycogen in the liver of rats. Unconjugated cortisol and coricosterone in plasma were determined by a fluorometric method.[9] Blood glucose was estimated by the *o*-toluidine method.[10] Liver glycogen was precipitated with alcohol,[11] then estimated as glucose with the *o*-toluidine method.[10] Rats were injected intraperitoneally with cAMP (100 mg/kg; ○——○) or with theophylline (50 mg/kg; ●——●). Each point represents the mean of at least three samples with S.E.

rats; 30 min after injection, the blood glucose level was 120 ± 5·0 mg/100 ml as compared with 90 ± 4·8 mg/100 ml in control rats. However, the level returned to normal rather rapidly within 60 min. Furthermore, theophylline did not augment the hyperglycemic effect of cAMP. This acute hyperglycemia induced by cAMP might be due to the direct effect of cAMP on glycogenolysis in the liver, because hepatic glycogen decreased in response to the increase in blood glycose, while no significant change in the plasma level of corticoids was observed after the administration of cAMP (100 mg/kg). Theophylline did not augment the effects of cAMP on the level of hepatic glycogen or plasma corticoids as well.

DISCUSSION

The data in this paper indicated that cAMP, which had been proved to suppress effectively the increases in vascular permeability and cutaneous histamine induced by PP_i and ATP in rats,[1] was also inhibitory to several other experimental inflammations of both the acute and chronic type when rats were pretreated with this nucleotide administered through various routes.

cAMP was effective in suppressing carrageenin-induced edema in the hind paws of rats, not only by intraperitoneal administration as reported by Bertelli *et al.*,[2] but also by oral and subcutaneous administration. In addition, the intraperitoneal injection of cAMP effectively suppressed the increase in vascular permeability induced by

thermal injury, exogenous PP_i or histamine, but it suppressed less effectively the increase induced by bradykinin.

Though the mechanism of the formation of carrageenin-induced edema is not yet clear, Van Arman *et al.*[7] suggested its mediation by bradykinin, mainly because histamine did not cause swelling in the hind paws of rats, and cyproheptadine, an antagonist to histamine and serotonin, was not effective in suppressing swelling induced by carrageenin as well as bradykinin. However, we did not observe the suppressive effect of cAMP, by either local subcutaneous[1] or systemic administration (Table 4) on the increase in vascular permeability induced by exogenous bradykinin, in spite of the suppressive effect of this nucleotide on carrageenin-induced edema.

Besides anti-edematic activity, cAMP also showed a similar dose-dependent inhibitory action in the formation of granuloma induced by carrageenin, a more chronic type of inflammation, when administered intraperitoneally or directly into granuloma. However, on preformed carrageenin-induced granuloma, lower doses of cAMP (1 or 10 mg, into granuloma), which were enough to suppress granuloma formation, had an apparent stimulatory effect. The reason for this is not yet clear.

Inhibitory potencies of cAMP on the various types of experimental inflammations used here were augmented by the presence of theophylline, as observed by Bertelli *et al.*[2] with rat plantarnal edema and dye leakage in rat skin induced by various phlogistins. Aminophylline (theophylline compound with ethylendiamine) given orally has been reported to exhibit its own anti-inflammatory activity on rat plantarnal edema induced by carrageenin.[14] Furthermore, potentiation of a hormonal effect by theophylline, e.g. increase of cardiac inotropic action of norepinephrine by theophylline,[15] is well known. However, Levine and Vogel[16] reported that pretreatment of dogs with theophylline did not alter cardiac responses fo cAMP, which mimics the action of catecholamines. Therefore, theophylline in our study apparently exerted its own anti-inflammatory action and also augmented the action of cAMP by inhibiting cAMP phosphodiesterase, as reported by Butcher and Sutherland.[17]

Since cAMP was equally as effective as N^6,$O^{2'}$-dibutyryl cAMP (DBcAMP) in suppressing PP_i-induced inflammation and histamine release from isolated mast cells,[1] we used the unsubstituted cAMP in the present study. DBcAMP has been reported to be generally more potent than the parent compound in most of the intact cell systems studied, probably due to its greater resistance to hydrolysis by cAMP phosphodiesterase and its higher penetration rate through cell membranes. However, cAMP has been found to be more effective than DBcAMP in several systems, e.g. in the activation of glycogen phosphorylase in dog liver extract,[18] in the stimulation of gluconeogenesis from lactate in isolated perfused rat kidney,[19] and in the inhibition of growth of cultured L-cells.[20] Exogenous cAMP entering cells might be degraded fairly rapidly, as observed by Heersche *et al.*[21] in the incubation *in vitro* of labeled cAMP with fetal rat calvaria. Then, the effectiveness of cAMP in suppressing acute inflammations induced 30 min or 1 hr later by various phlogistins may indicate that only a transient elevation of the intracellular concentration of cAMP above the critical level in certain tissues is required for its subsequent exertion of biological functions. In this connection, Jost *et al.*[22] observed a rapid and transient increase in the concentration of hepatic cAMP before the induction of serine dehydratase in rat liver by glucagon intraperitoneally administered.

Further investigations of the anti-inflammatory activity of DBcAMP seem to be

necessary to understand the complex function of cAMP in the inflammatory process, not only because of the augmentative effects of theophylline on cAMP, but also because of the following facts reported: (1) Heersche *et al.*[21] observed a more potent inhibitory effect of DBcAMP than of theophylline on cAMP phosphodiesterase in bone tissue. (2) According to Posternak *et al.*[18] removal of at least one butyryl group from DBcAMP (to N^6-monobutyryl cAMP) seemed to be necessary prior to its activation of glycogen phosphorylase in dog liver extract, while Blecher *et al.*[23] reported the stimulation of lipolysis in isolated rat fat cells by DBcAMP itself without any removal of butyryl groups. (3) According to Rudack *et al.*,[24] cAMP was as effective as glucagon in preventing the induction of glucose 6-phosphate dehydrogenase in livers of rats fasted and then refed a high carbohydrate, non-fat diet, while DBcAMP, though effective, decreased the amount of diet consumed. Furthermore, the susceptibility of cAMP to hydrolytic degradation might be a desirable property in regulating the cellular concentration of this nucleotide.

Though the mechanism of anti-inflammatory action of cAMP is not clear, Bertelli *et al.*[2] suggested that cAMP would inhibit inflammation by increasing the production of glucocorticoids or by causing hyperglycemia. Several investigators have demonstrated that cAMP stimulates, as effectively as ACTH, the formation of corticosteroids in isolated dog adrenals by direct arterial perfusion of this nucleotide,[25] in adrenals of hypophysectomized rats by femoral infusion,[26] and also in adrenal slices of rats,[25] hypophysectomized rats,[27] guinea-pigs,[25] and human beings[28] incubated with cAMP *in vitro*. However, under our experimental conditions, intraperitoneal administration of cAMP alone or with theophylline (100 mg/kg each) did not significantly alter the steroid concentration of rat plasma. Imura *et al.*[26] also reported that, in intact rats, intravenous infusion of cAMP did not bring about any greater increase in plasma corticosterone than did saline infusion. From these results and from our observation of the suppressive effect of cAMP on edema formation in adrenalectomized rats, it seems unlikely that cAMP elicits its anti-inflammatory effect solely by increasing the production of corticosteroids.

Inflammation induced by dextran or egg-white has been reported to be markedly inhibited in alloxan-diabetic rats[29,30] and in normal rats made hyperglycemic by glucose overdosage.[31] Hyperglycemic effects of cAMP and its dibutyryl derivative have been reported in rats,[32] dogs,[16,33] human beings[34] and other animals,[35] and also in perfused rat liver[32,36] and rat heart.[37] Hyperglycemia induced by cAMP, alone or with theophylline (each 100 mg/kg, i.p.), was only 30–50 per cent above the control level in 60 min, which corresponds well with the changes in liver glycogen (Fig. 2). Therefore, it is unlikely that this slight and transient hyperglycemia inhibits inflammations, since prolonged hyperglycemia was required to suppress the inflammation in alloxan-diabetes[29] and in glucose overdosage.[31] As indicated in the elevation of heart rate observed within seconds after administration by Bergen *et al.*,[33] cAMP might produce a more direct action on the inflammatory process.

According to Weissmann *et al.*,[38] cAMP, DBcAMP and prostaglandin, which stimulated adenyl cyclase of human leukocytes,[39] inhibited the phagocytic release of lysosomal hydrolases by human polymorphonuclear leukocytes or by mouse macrophages. (A similar inhibition by cAMP of the release of β-glucuronidase from human leukocytes was also reported by May *et al.*[40] Theophylline augmented the inhibitory effects of cAMP also in these cases. Considering the important role of lysosomes in

inflammatory processes, our results may suggest a possible regulatory effect of cAMP on the function of phagocytic cells in injured regions, which requires further investigation.

REFERENCES

1. A. ICHIKAWA, H. HAYASHI, M. MINAMI and K. TOMITA, *Biochem. Pharmac.* **21,** 317 (1972).
2. A. BERTELLI, A. CERRATI, A. G. PERAZZOLI and M. A. ROSSANO, *Atti Acad. med. Lambara* **21,** 601 (1966).
3. C. A. WINTER, E. A. RISLEY and G. W. NUSS, *Proc. Soc. exp. Biol. Med.* **111,** 544 (1962).
4. W. G. SPECTOR and D. A. WILLOGHBY, *The pharmacology of inflammation*, p. 111. English University Press, London (1968).
5. D. S. JACKSON, in *Advances in biology of skin* (Ed. E. MONTAGNA and R. E. BILLINGHAM), Vol. 5, p. 30. Macmillan, New York (1964).
6. C. J. E. NIEMEGEERS, F. J. VERBRUGGEN and P. A. J. JANSSEN, *J. Pharm. Pharmac.* **16,** 810 (1964).
7. C. G. VAN ARMAN, A. J. BEGANY, L. M. MILLER and H. H. PLESS, *J. Pharmac. exp. Ther.* **150,** 328 (1965).
8. M. FUKUHARA and S. TSURUFUJI, *Biochem. Pharmac.* **18,** 475 (1969).
9. W. G. SPECTOR and D. A. WILLOUGHBY, *J. Path. Bact.* **78,** 121 (1959).
10. V. L. BEACH and B. G. STEINETZ, *J. Pharmac. exp. Ther.* **131,** 400 (1961).
11. E. NIELSEN and V. H. ASFELDT, *Scand. J. clin. Lab. Invest.* **20,** 185 (1967).
12. E. HULTMAN, *Nature, Lond.* **183,** 108 (1959).
13. C. A. GOOD, H. KRAMER and M. SOMOGYI, *J. biol. Chem.* **100,** 485 (1933).
14. G. R. MCKINNEY and P. M. LISH, *Proc. Soc. exp. Biol. Med.* **117,** 280 (1964).
15. T. W. RALL and T. C. WEST, *J. Pharmac. exp. Ther.* **139,** 269 (1963).
16. R. A. LEVINE and J. A. VOGEL, *J. Pharmac. exp. Ther.* **151,** 262 (1966).
17. R. W. BUTCHER and E. W. SUTHERLAND, *J. biol. Chem.* **237,** 1244 (1962).
18. TH. POSTERNAK, E. W. SUTHERLAND and W. F. HENION, *Biochim. biophys. Acta* **65,** 558 (1962).
19. R. H. BOWMAN, *J. biol. Chem.* **245,** 1604 (1970).
20. W. L. RYAN and M. L. HEIDRICK, *Science, N.Y.* **162,** 1484 (1964).
21. J. N. M. HEERSCHE, S. A. FEDAK and G. D. AURBACH, *J. biol. Chem.* **246,** 6770 (1971).
22. J.-P. JOST, A. HSIE, S. D. HUGHES and L. RYAN, *J. biol. Chem.* **245,** 351 (1970).
23. M. BLECHER, J. T. RO'ANE and P. D. FLYNN, *J. biol. Chem.* **245,** 1867 (1970).
24. D. RUDAK, B. DAVIE and D. HOLTON, *J. biol. Chem.* **246,** 7823 (1971).
25. J. G. HILTON, O. R. KRUESI, R. I. NEDELJKOVIC and L. F. SCIAN, *Endocrinology* **68,** 908 (1961).
26. H. IMURA, S. MATSUKURA, H. MATSUYAMA, T. SETSUDA and T. MIYAKE, *Endocrinology* **76,** 933 (1965).
27. R. C. HAYNES, JR., S. B. KORITZ and F. G. PÉRON, *J. biol. Chem.* **234,** 1421 (1959).
28. G. P. STUDZINSKI and J. K. GRANT, *Nature, Lond.* **193,** 1075 (1962).
29. V. W. ADAMKIEWICZ and L. M. ADAMKIEWICZ, *Am. J. Physiol.* **197,** 377 (1959).
30. A. GOTH, W. L. NASH, M. NAGLER and J. HOLMAN, *Am. J. Physiol.* **191,** 25 (1957).
31. V. W. ADAMKIEWICA and L. M. ADAMKIEWICZ, *Am. J. Physiol.* **198,** 51 (1960).
32. G. NORTHROP and R. E. PARKS, JR., *Biochem. Pharmac.* **13,** 120 (1964).
33. S. S. BERGEN, JR., J. G. HILTON and T. B. VAN ITALLIE, *Endocrinology* **79,** 1065 (1966).
34. R. A. LEVINE, *Clin. Pharmac. Ther.* **11,** 238 (1970).
35. R. A. LEVINE and J. A. VOGEL, *Nature, Lond.* **207,** 987 (1965).
36. R. A. LEVINE and S. E. LEWIS, *Biochem. Pharmac.* **18,** 15 (1969).
37. K. AHREN, A. HJALMARSON and O. ISAKSSON, *Acta physiol. Scand.* **82,** 79 (1971).
38. G. WEISSMANN, P. DUKOR and R. B. ZURIER, *Nature New Biol.* **231,** 131 (1971).
39. R. E. SCOTT, *Blood* **35,** 514 (1970).
40. C. D. MAY, B. B. LEVINE and G. WEISSMANN, *Proc. Soc. exp. Biol. Med.* **133,** 758 (1970).