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Inotropic and Chronotropic Actions of 2-Substituted and 8-Substituted Derivatives of Adenosine 3′,5′-Cyclic Monophosphate[1]

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We synthesized several 2- and 8-substituted derivatives of adenosine 3′,5′-cyclic monophosphate (c-AMP), and examined the effects of these compounds on the isolated atrial muscle preparation of the guinea pig. We found that several 8-substituted c-AMPs could produce definite positive inotropic and chronotropic effects, even when applied from outside the cell, while all the 2-substituted c-AMPs produced negative inotropic and chronotropic effects. Pretreatment of the preparation with aminophylline resulted in a potentiation of the positive inotropic and chronotropic effects of 8-substituted c-AMPs, while the negative inotropic and chronotropic effects of 2-substituted c-AMPs were abolished. The mechanisms by which these effects were produced are discussed.

Keywords——cyclic AMP derivative; inotropic action; chronotropic action; cardiac contractility; guinea pig

During the past few years, adenosine 3′,5′-cyclic monophosphate (c-AMP) has been identified as the second messenger of various hormones.[3] Compounds which affect the level of c-AMP in cardiac tissue have therefore been tested for effects on cardiac contractility. Attempts have also been made to demonstrate positive intropic effects of c-AMP itself, but without success.[4] Among various c-AMP derivatives, only dibutyryl c-AMP was found to produce a positive intropic effect in isolated perfused rat heart and cat papillary muscle.[5] We synthesized 8-substituted c-AMP derivatives by the method of Muneyama[6] and 2-substituted c-AMP derivatives by deblocking of the etheno residue from 2-substituted 1,N^6-etheno c-AMP derivatives.[7]

This paper deals with the effects of several 2- and 8-substituted c-AMP derivatives on isolated atrial strips of the guinea pig.

Experimental

Animals——Albino guinea pigs of either sex weighing between 300—550 g were sacrificed by means of a blow on the head. The atria were quickly excised, freed from excess tissues and divided into the right and left halves. The right atrium, which retained spontaneous rhythm, was used as a whole to asses the chrono-

1) A preliminary account of this study has appeared in *Japan. J. Pharmacol.*, **25**, 601 (1975) and a part of this paper was presented at the North Area Regional Meeting of the Japanese Pharmacological Society, October 1978.
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3) P. Greengard, R. Paoletti, and G.A. Robison (ed.), "Advances in Cyclic Nucleotide Research," Vol. 1, Raven Press, New York, 1972.
4) M.L. Entman, "Advances in Cyclic Nucleotide Research," Vol. 4, ed. by P. Greengard and G.A. Robison, Raven Press, New York, 1974, pp. 163—193.
5) *a*) K. Ahren, A. Hjalmarson, and O. Isaksson, *Acta Physiologica Scandinavica*, **82**, 79 (1971); *b*) C.L. Skelton, G.S. Levey, and S.E. Epstein, *Circulation Res.*, **26**, 35 (1971).
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7) *a*) N. Yamaji, K. Suda, Y. Onoue, and M. Kato, *Chem. Pharm. Bull.*, **25**, 2339 (1977); *b*) N. Yamaji, K. Tahara, and M. Kato, *ibid.*, **26**, 2391 (1978).

tropic action of the drugs. Atrial preparations were mounted in a 20 ml organ bath. The initial resting tension was set at 0.2—0.3 g, and the rate of spontaneous contraction of this preparation was recorded on an ink-pen oscillograph by means of a linearly-recording tachograph (Nihon Kohden RT-5). The left atrium was used to evaluate the inotropic effect. This preparation was stimulated electrically using a square-wave pulse stimulator (Nihon Kohden MSE-40) at a frequency of 1 Hz with voltages approx. 30% above the threshold (duration=1 msec). The resting tension of the preparation was kept at 0.2 g throughout the course of the experiment, and the contractile tension was recorded on an ink-pen oscillograph with a strain-gauge transducer and a carrier-amplifier. The bathing solution used was Krebs-Henseleit's solution of the following composition: NaCl 118 mM, $CaCl_2$ 2.5 mM, $NaHCO_3$ 24.9 mM, $MgSO_4$ 1.2 mM, KH_2PO_4 1.2 mM, and glucose 12 mM. The temperature of the solution was maintained at 32±0.3°. The solution was aerated with a mixture of 95% O_2 and 5% CO_2. After dissection, all preparations were allowed to equilibrate for one hour prior to addition of any drug.

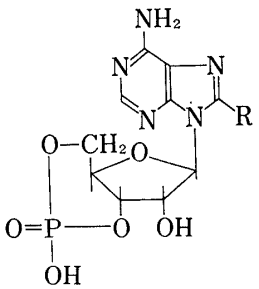
Drug——The drugs used were as follows: 8-thio c-AMP, 8-oxy c-AMP, 8-amino c-AMP, 8-bromo c-AMP, 8-azido c-AMP, 8-methylthio c-AMP, 8-benzylthio c-AMP, 8-methoxy c-AMP, 8-dimethylamino c-AMP, 2-oxy c-AMP, 2-amino c-AMP, 2-fluoro c-AMP, 2-chloro c-AMP, 2-bromo c-AMP, 2-iodo c-AMP, 2-azido c-AMP, 2-methylthio c-AMP, 2-methoxy c-AMP, 2-dimethylamino c-AMP, and N^6,2′-O dibutyryl c-AMP. 8-Substituted c-AMP derivatives and 2-substituted c-AMP derivatives were synthesized as the free acids by the method of Muneyama[6] and by the method described in the preceding papers.[7] Because of the low solubility of these compounds, they were dissolved in Krebs-Henseleit's solution and administered to the preparation by replacing 2—5 ml of the bathing solution. N^6,2′-O dibutyryl c-AMP was supplied by Daiichi Pharmaceutical Co., Tokyo, Japan, as the Na salt, and was dissolved in redistilled H_2O. It was injected into the bathing solution (volume: less than 1.6 ml).

Result and Discussion

Table I summarizes the results obtained with nine 8-substituted derivatives of c-AMP. Eight of the derivatives produced definite positive inotropic and chronotropic effects.

As exemplified in Fig. 1, which shows the positive inotropic effects of 3 representative compounds, the effects were dose-dependent in the range of 10^{-4}—5×10^{-4} g/ml and were fully reversible. Higher doses were not tested because of the low solubility of these compounds in Krebs-Henseleit's solution. With some compounds, the positive inotorpic effects exceeded the maximum effects obtained with 10^{-7} M isoproterenol (Isp). To illustrate the time course of the effects of 8-eubstituted c-AMP derivatives, the positive inotropic effect of 0.5 mg/ml of 8-thio c-AMP is depicted in Fig. 2 in comparison with that of 2 mg/ml dibutyryl c-AMP.

Table I. Cardiac Actions of 8-Substituted Cyclic AMP Derivatives (0.5 mg/ml) in Guinea Pig Atria

Chemical structure	R=	Positive chronotropic	Positive inotropic
	SH	79.5±4.2	134.4±20.3
	OH	84.7±0.3	119.3±12.3
	$SCH_2C_6H_5$	84.2±6.7	89.9±3.6
	N_3	68.4±2.1	47.9±7.6
	SCH_3	59.8±3.9	40.2±3.3
	OCH_3	56.2±3.1	28.9±9.7
	$N(CH_3)_2$	31.8±12.8	20.1±1.1
	Br	62.3±6.2	27.9±11.6
	NH_2	variable*	variable*

% of the maximum responce to Isp. Mean±S.E. (n=4).
* Either a positive (2/4) or a negative effect (2/4) was observed.

Just as in the case of the positive inotropic effect of dibutyryl c-AMP, the effect of the 8-thio c-AMP appeared slowly, taking more than 20 minutes to attain a steady maximum. Because of the low solubility of 8-thio c-AMP, we did not employ a higher concentration of this compound. However, unlike dibutyryl c-AMP, which invariably produced initial negative

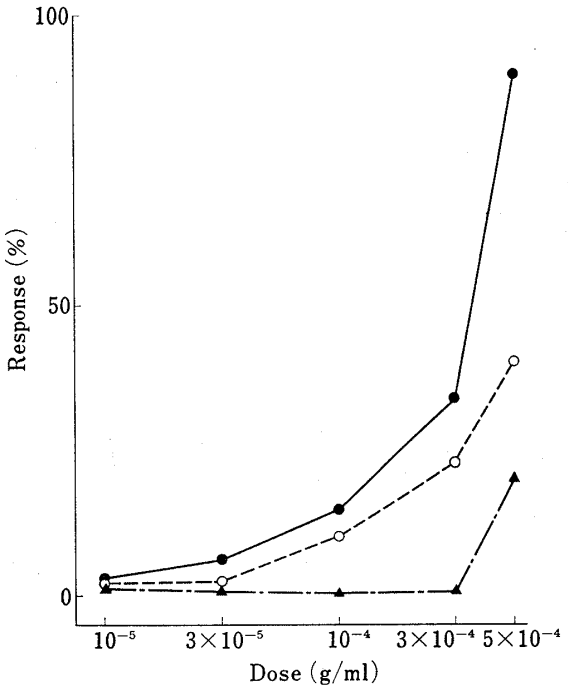


Fig. 1. Positive Inotropic Effects of 8-Substituted c-AMP Derivatives

Responces are expressed as % of the maximum responce to Isp. (n=4).
—●—: 8-$SCH_2C_6H_5$,
---○---: 8-SCH_3,
---▲---: 8-$N(CH_3)_2$.

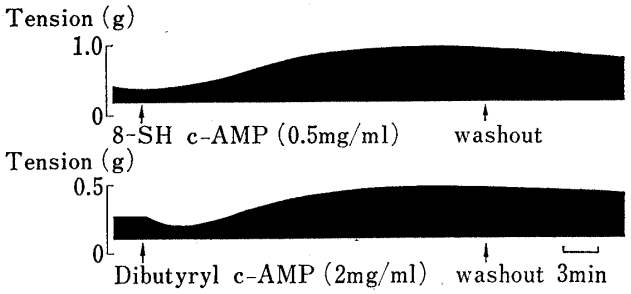


Fig. 2. Positive Inotropic Effects of 8-SH Cyclic AMP (8-SH c-AMP) and Dibutyryl Cyclic AMP (Dibutyryl c-AMP) in the isolated Left Atria of the Guinea Pigs

Atria were driven with square-wave pulses using an electronic stimulator (Frequency: 1 Hz; Duration: 1 msec).

Table II. Negative Chronotropic Action of 2-Substituted Cyclic AMP Derivatives in Guinea Pig Atria

Residue	Dose (g/ml)								ID_{50} (g/ml)
	3×10^{-8}	10^{-7}	3×10^{-7}	10^{-6}	3×10^{-6}	10^{-5}	3×10^{-5}	10^{-4}	
Cl	8.30± 3.53[a)]	25.25± 10.19	46.55± 10.19	74.78± 13.31	89.83± 10.18	98.15± 1.85			3.4×10^{-7}
N_3	1.60± 0.70	4.20± 1.33	11.60± 3.53	32.08± 4.54	48.28± 4.02	70.98± 7.56	83.83± 9.35		3.3×10^{-6}
F	2.00± 1.00	5.25± 2.67	9.35± 5.02	17.85± 6.43	35.38± 4.51	63.30± 12.44	86.27± 13.73		5.6×10^{-6}
Br	1.65± 0.25	4.10± 0.90	8.15± 2.69	23.78± 3.83	38.30± 3.35	50.90± 3.70			9.0×10^{-6}
OH	0.88± 0.54	2.80± 1.08	4.50± 1.52	10.35± 3.45	26.88± 5.86	46.70± 8.62	85.73± 6.89	95.30± 0.60	1.2×10^{-5}
I	0.98± 1.18	1.00± 1.98	−3.25± 4.94	2.50± 2.31	5.90± 2.34	19.93± 7.30	35.00± 7.10	54.05± 6.91	7.6×10^{-5}
OCH_3	0.63± 0.63	1.25± 0.75	0.55± 0.38	0.80± 0.46	1.48± 1.48	5.18± 2.39	13.78± 6.30	27.85± 9.86	$>10^{-4}$
SCH_3	0	0	0.61± 0.61	−0.33± 1.04	−0.18± 1.49	1.50± 2.23	7.05± 3.54	13.60± 4.72	$>10^{-4}$
NH_2	0	−1.25± 1.00	−1.55± 1.00	−2.63± 2.00	−0.93± 3.01	−0.50± 4.40	0.08± 6.16	3.08± 8.30	$>10^{-4}$
$N(CH_3)_2$	−1.14± 0.09	−1.23± 0.77	−2.10± 1.17	−2.10± 1.35	−3.08± 2.03	−2.83± 2.65	−2.00± 3.10	−0.65± 3.34	$>10^{-4}$

a) B/A×100 (%) Mean±S.E. (n=4)

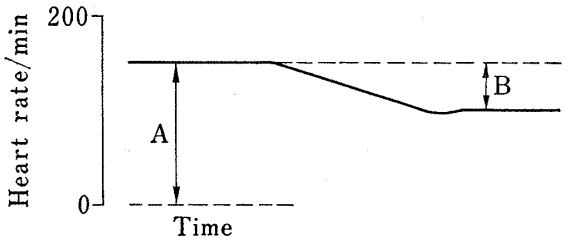
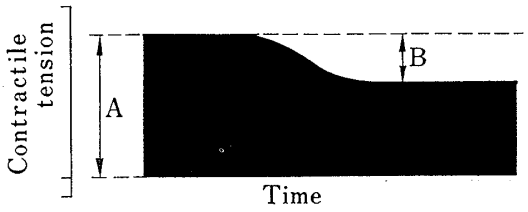


Table III. Negative Inotropic Action of 2-Substituted Cyclic AMP Derivatives in Guinea Pig Atria

Residue	Dose (g/ml)								ID_{50} (g/ml)
	3×10^{-8}	10^{-7}	3×10^{-7}	10^{-6}	3×10^{-6}	10^{-5}	3×10^{-5}	10^{-4}	
Cl	12.00± 3.44[a)]	27.02± 5.81	48.25± 4.98	66.18± 2.91	76.11± 1.27	78.84± 1.67			3.3×10^{-7}
N_3	2.48± 1.44	9.03± 3.12	25.60± 4.33	50.53± 6.59	71.73± 4.26	81.75± 2.39	85.03± 2.09	86.95± 1.38	9.8×10^{-7}
Br	5.95± 1.75	22.70± 8.10	22.75± 5.40	43.00± 5.00	61.83± 6.51	72.30± 6.60			1.5×10^{-6}
OH	0.60± 0.36	3.08± 1.03	5.13± 2.40	14.50± 5.48	54.20± 9.80	71.80± 4.26	83.00± 2.33	86.10± 2.27	2.7×10^{-6}
F	3.05± 1.20	5.78± 1.48	14.28± 1.88	32.55± 2.59	48.85± 2.33	66.80± 1.35	76.00± 11.58	82.20± 1.20	3.2×10^{-6}
I	2.08± 2.08	1.03± 0.59	3.83± 1.53	10.08± 3.34	23.05± 7.44	43.85± 9.24	66.25± 7.09	80.55± 3.29	1.4×10^{-5}
OCH_3	−1.32± 1.32	−2.65± 2.65	−3.90± 3.90	−3.28± 6.20	0.48± 7.14	11.10± 8.67	27.05± 10.30	58.63± 3.60	7.2×10^{-5}
SCH_3	2.73± 1.62	2.75± 2.29	3.25± 2.60	6.05± 4.32	8.58± 4.70	12.33± 4.40	25.73± 6.56	39.75± 5.41	$>10^{-4}$
NH_2	−3.30± 3.30	−1.53± 4.10	−3.10± 4.58	−1.10± 3.25	7.13± 2.85	9.63± 5.10	14.60± 5.88	23.18± 7.36	$>10^{-4}$
$N(CH_3)_2$	0.05± 0.94	2.53± 1.46	5.48± 2.27	9.50± 2.82	10.70± 2.71	14.70± 3.74	19.23± 3.52	19.10± 5.71	$>10^{-4}$

a) B/A×100 (%) Mean±S.E. ($n=4$)



effects, as shown in Fig. 2, such a negative phase was only rarely observed with 8-substituted compounds.

Table II and III summarize the reults obtained with ten 2-ubstituted derivatives of c-AMP.

Seven of these derivatives produced definite negative inotropic and chronotropic effects. The negative chronotropic effects were dose-dependent in the range of 3×10^{-8}—10^{-5} g/ml for 2-chloro c-AMP, 3×10^{-7}—3×10^{-5} for 2-azido, 2-fluoro, 2-bromo and 2-hydroxy c-AMP and 3×10^{-6}—10^{-4} for 2-iodo and 2-methoxy c-AMP.

The negative inotropic effects were also dose-dependent in the range of 3×10^{-8}—10^{-5} for 2-chloro c-AMP, 10^{-7}—10^{-5} for 2-azido c-AMP, 3×10^{-7}—3×10^{-5} for 2-bromo and 2-fluoro c-AMP, 10^{-6}—3×10^{-5} for 2-iodo c-AMP and 3×10^{-6}—10^{-4} for 2-methoxy c-AMP. The order of potency was halogen>hydroxy>amine, and this is in accordance with the order of electron-withdrawing effects of the substituents. Thus, electron deficiency of the adenine moiety may be responsible for the negative chronotropic and inotropic effects. This assumption is further supported by the finding that 2-methoxy c-AMP was much less potent than 2-hydroxy c-AMP, the corresponding free compound. As a preliminary step in elucidating the underlying mechanism of the positive inotropic and chronotropic effects of 8-substituted c-AMP derivatives and the negative inotropic and chronotropic effects of 2-substituted c-AMP derivatives, we examined the effects of pretreatment of the preparation with aminophylline. Pretreatment of the preparation with 10^{-4} M aminophylline for 60 min resulted in a potentiation of the positive inotropic effects of 8-thio c-AMP, 8-benzylthio c-AMP, and dibutyryl c-AMP, but a depression of the negative inotropic effects of 2-substituted c-AMP derivatives. However, even after aminophylline treatment no positive effects were observed with the 2-

substituted c-AMP derivatives.

It may be reasonable to assume that the positive inotropic and chronotropic effects of 8-substituted c-AMPs are due to an accumulation of c-AMP within the cell, as postulated in the case of dibutyryl c-AMP.[8] However, since the potentiation by aminophylline of the effects of 8-substituted c-AMPs was not as marked as that of dibutyryl c-AMP, the possibility that 8-substituted compounds act through inhibition of the phosphodiesterase cannot be ruled out.

As the physical properties of 2-substituted c-AMP derivatives and 8-substituted c-AMP derivatives are very similar, the penetrability of both derivatives through cell membranes may be much the same. Therefore, the completely opposite effects produced by these two derivatives may be due to different behavior of these two kinds of derivatives towards phosphodiesterase. Whereas 2-substituted c-AMP derivatives are easily hydrolyzed to the corresponding 5′-AMP derivatives by phosphodiesterase,[9] 8-substituted derivatives (except for 8-amino c-AMP) are resistant to degradetion by phosphodiesterase.[6] The fact that the effects of 8-amino c-AMP were variable (Table I) is compatible with this consideration, since 8-amino c-AMP is the only 8-substituted derivatives that can be degraded to some degree by phosphodiesterase.

High susceptibility to phosphodiesterase as well as poor membrane penetrability may explain the lack of positive inotropic and chronotropic effects of exogenous c-AMP itself.

At any rate, we now have several more derivatives of c-AMP which can produce definite positive inotropic and chronotropic effects, even when supplied from outside the cell, and these may well prove to be useful pharmacological tools.

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