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INTERACTION BETWEEN ADENOSINE AND CATECHOLAMINES ON CYCLIC AMP ACCUMULATION IN GUINEA PIG VENTRICULAR MYOCARDIUM

MINTA HUANG and GEORGE I. DRUMMOND
Biochemistry Group, Department of Chemistry, University of Calgary, T2N 1N4, Canada

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Abstract—Adenosine stimulated cyclic AMP accumulation in guinea pig ventricular slice preparations. Stimulation produced by adenosine and isoproterenol combined was essentially the sum of the two individual responses. In contrast, the response produced by adenosine and epinephrine or adenosine and norepinephrine was lower than that produced by the nucleoside alone. In the absence or presence of adenosine, propranolol decreased epinephrine- or norepinephrine-mediated accumulation of cyclic AMP below control levels, whereas phentolamine increased these responses. Methoxamine reduced cyclic AMP accumulation mediated by isoproterenol or adenosine. The results are interpreted to indicate that alpha and beta adrenergic agents have opposing effects on cyclic AMP formation in this preparation, alpha receptor stimulation acting to decrease cyclic AMP levels. The effect of alpha receptor activation was particularly pronounced when cyclic AMP levels were elevated by adenosine.

In a recent study [1] we showed that adenosine produced a rapid and dose-dependent increase in cyclic AMP levels in guinea pig ventricular slice preparations. The stimulatory effect of adenosine was potentiated by several phosphodiesterase inhibitors and by agents which prevent uptake of the nucleoside into myocardial cells and was inhibited by theophylline. In tissue slice preparations from guinea pig cerebral cortex, where the ability of adenosine to stimulate cyclic AMP formation was first discovered [2], the nucleoside and norepinephrine act synergistically to elevate cyclic AMP levels [2–7]. The mechanism of this interaction is not understood; Sattin *et al.* [6] have proposed that adenosine and a catecholamine may act either as dependent or independent coactivators of adenylate cyclase. The ability of beta adrenergic amines to stimulate adenylate cyclase activity and to increase cyclic AMP levels in the heart is well known. The action of alpha adrenergic agents on the heart is less well understood, but it has been proposed [8] that alpha and beta adrenergic receptors might have opposing actions on cyclic AMP formation. We have examined possible interactions between adenosine and catecholamines on cyclic AMP accumulation in tissue slice preparations of the ventricular myocardium. Data presented show that, while beta receptor agonists act in an additive manner with adenosine, alpha receptor agonists act antagonistically with the nucleoside and also with the beta adrenergic agonist, isoproterenol.

MATERIALS AND METHODS

Ro 20-1724* was obtained through the courtesy of Dr. H. Sheppard, Hoffmann-La Roche Inc., Nutley, NJ, U.S.A. Phentolamine was provided by Ciba Co.; (−)isoproterenol bitartrate, (−)epinephrine bitartrate, norepinephrine HCl, and adenosine were purchased from Sigma Chemical Co., St. Louis, MO; methoxamine HCl was obtained from Burroughs Wellcome Co., Research Triangle Park, NC, U.S.A. Female guinea pigs (300–450 g) were purchased from Canadian Breeders, Ltd., Montreal, Canada.

Experimental procedure. Preparation of ventricular slices, composition of incubation medium, conditions for incubation, purification of tissue extracts and assays for cyclic AMP and for protein were as described previously [1]. Ventricular muscle from three hearts was used for each experiment; this provided sufficient material for 18–20 incubations.

In most experiments, accumulation of cyclic AMP was measured in the presence of Ro 20-1724 (0.1 mM). The phosphodiesterase inhibitor was added 15 sec prior to the addition of other test substances which were prepared in a mixture. Incubations were conducted for 3–5 min at 37° and were terminated by homogenizing the slices in 1 ml of 8% trichloroacetic acid at 4° [1].

RESULTS

Effect of adenosine and catecholamines on cyclic AMP accumulation. In accord with our previous results [1], 0.1 mM adenosine increased cyclic AMP accumulation in guinea pig ventricular slices about 4-fold (Table 1); Ro 20-1724, which elevated the cyclic nucleotide content by inhibiting phosphodiesterase, potentiated the action of adenosine. Epinephrine and isoproterenol did not augment cyclic AMP levels in this preparation unless a phosphodiesterase inhibitor was present. In the presence of 0.1 mM Ro 20-1724, isoproterenol and epinephrine increased cyclic AMP levels from 3.4 (control) to 9.9 and 6.5 pmoles/mg of protein, respectively (Table 1, column 1); a maximal effect was obtained after 3 min of incubation with 0.01 mM of each. Norepinephrine had no stimulatory

*The following abbreviation has been used: Ro 20-1724, DL-4-(3-butoxy-4-methoxy-benzyl)-2-imidazolidinone.

Table 1. Effect of catecholamines and adenosine on cyclic AMP accumulation in guinea pig ventricular slices*

Additions	Concn (mM)	Cyclic AMP (pmoles/mg protein) I No blocking agent	II + Phentolamine (0.01 mM)	III + Propranolol (0.01 mM)
None		0.94 ± 0.09 (11)	0.97 ± 0.12† (3)	0.63 ± 0.12† (3)
Adenosine	0.10	4.0 ± 0.23 (10)	3.5 ± 0.34† (4)	3.6 ± 0.33† (4)
Ro 20-1724	0.10	3.4 ± 0.24 (10)	3.6 ± 0.46† (3)	4.4 ± 0.34‡ (4)
Adenosine	0.10			
Ro 20-1724	0.10	13.2 ± 0.70 (10)	11.2 ± 0.89† (4)	12.0 ± 1.07† (4)
Isoproterenol	0.01			
Ro 20-1724	0.10	9.9 ± 0.42 (5)	11.1 ± 0.44† (3)	3.9 ± 0.26§ (3)
Adenosine	0.10			
Isoproterenol	0.01			
Ro 20-1724	0.10	17.0 ± 1.34 (4)	17.1 ± 1.57† (3)	12.0 ± 1.0‖ (4)
Epinephrine	0.01			
Ro 20-1724	0.10	6.5 ± 0.29 (11)	8.2 ± 0.39‡ (4)	1.8 ± 0.09§ (4)
Epinephrine	0.01			
Adenosine	0.10			
Ro 20-1724	0.10	10.0 ± 0.24¶ (8)	15.5 ± 0.78§ (3)	5.5 ± 0.36§ (4)
Norepinephrine	0.01			
Ro 20-1724	0.10	3.5 ± 0.1 (3)	4.5 ± 0.02§ (3)	2.0 ± 0.08§ (3)
Norepinephrine	0.01			
Adenosine	0.10			
Ro 20-1724	0.10	8.0 ± 0.44** (6)	13.0 ± 0.33§ (4)	6.5 ± 0.19‖ (4)

* Values are expressed as means ± S. E. M. from the number of experiments indicated in parentheses. The significance of a difference from a corresponding value in the absence of antagonists was determined by Student's *t*-test and is indicated by the probabilities shown in footnotes †, ‡, § and ‖. The significance of a difference from a corresponding value in the absence of an appropriate catecholamine is indicated by the probabilities shown in footnotes ¶ and **.

† $P > 0.1$. § $P < 0.001$. ¶ $P < 0.002$.
‡ $P < 0.01$. ‖ $P < 0.05$. ** $P < 0.001$.

action at 0.01 mM in the presence of the phosphodiesterase inhibitor. When adenosine and isoproterenol were present together at 0.1 and 0.01 mM, respectively, cyclic nucleotide levels rose well above that produced by adenosine alone, and were approximately the sum of the two individual responses. In contrast, when epinephrine or norepinephrine was present with adenosine, cyclic AMP was reduced to levels significantly below that achieved with the nucleoside alone, i.e. from 13.2 (adenosine alone) to 10.0 and 8.0 pmoles/mg of protein, respectively (Table 1, column I). Since isoproterenol is a pure beta receptor agonist, and epinephrine and norepinephrine stimulate both alpha and beta receptors, the data suggest that, in the case of the latter two catecholamines, adenosine stimulation of cyclic AMP formation might be antagonized by alpha agonist activity. Thus, we examined the effects of the alpha and/or beta components of each of the catecholamines on adenosine-elicited accumulation of cyclic AMP. This was accomplished by using the alpha adrenergic blocking agent, phentolamine, or the beta adrenergic blocking agent, propranolol. Neither blocking agent (0.01 mM) significantly altered the basal level of cyclic AMP nor the adenosine-stimulated level in the absence or presence of Ro 20-1724 (Table 1, columns II and III). Ro 20-1724-stimulated cyclic AMP accumulation was unaffected by phentolamine but was slightly increased by propranolol. As expected, isoproterenol stimulation of cyclic AMP was completely abolished by propranolol, but unaffected by phentolamine. Similarly, propranolol reduced the combined effect of adenosine and isoproterenol to near that achieved with adenosine alone (compare 13.2 ± 0.7 with 12.0 ± 1.0), while phentolamine did not alter the effect of these two agonists combined. In marked contrast, when epinephrine was tested in the presence of propranolol, cyclic AMP was reduced below control levels (compare 4.4 ± 0.34 with 1.8 ± 0.09); when epinephrine was tested in the presence of phentolamine, cyclic AMP levels increased significantly above those achieved with the catecholamine alone (compare 6.5 ± 0.29 with 8.2 ± 0.39). These effects were even more pronounced when the tissue cyclic AMP level was elevated by adenosine. Thus, propranolol caused a drastic decrease in the combined effect of epinephrine and adenosine; the levels were reduced much below that achieved with adenosine alone (compare 13.2 ± 0.7 with 5.5 ± 0.36). As with epinephrine alone, phentolamine caused an increase in the combined effects of epinephrine and adenosine (10.0 ± 0.24 compared with 15.5 ± 0.78). The results with norepinephrine in the presence of either blocking agent were similar to those for epinephrine. Thus, propranolol reduced the levels achieved with this catecholamine alone, and phentolamine had a stimula-

Table 2. Effect of methoxamine on isoproterenol- and adenosine-mediated accumulation of cyclic AMP*

Agents	Concn (mM)	Cyclic AMP (pmoles/mg protein) No blocking agent	+ Phentolamine (0.01 mM)
Isoproterenol	0.01	9.9 ± 0.36 (4)	11.1 ± 0.44† (3)
Isoproterenol	0.01		
Methoxamine	0.1	7.5 ± 0.44‡ (4)	8.6 ± 0.16§ (4)
Adenosine	0.1	14.2 ± 0.66 (6)	12.0 ± 1.07‖ (4)
Adenosine	0.1		
Methoxamine	0.1	8.9 ± 0.32¶ (5)	11.2 ± 0.42** (5)

* All incubations contained 0.1 mM Ro 20-1724. Values are expressed as means ± S. E. M. from the number of experiments indicated in parentheses. The significance of a difference from a corresponding value in the absence of phentolamine was determined by Student's *t*-test and is indicated by the probabilities shown in footnotes †, §, ‖ and **. The significance of a difference from a corresponding value in the absence of methoxamine is indicated by the probabilities shown in footnotes ‡ and ¶.

§ $P < 0.02$. ¶ $P < 0.001$.
† $P > 0.1$. ‖ $P > 0.05$. ** $P < 0.002$.
‡ $P < 0.01$.

tory effect; when adenosine and norepinephrine were present together, propranolol significantly reduced the levels below that achieved in its absence, while phentolamine produced a striking increase, the level rising to that produced by adenosine alone. These data can be interpreted to indicate opposing effects of alpha and beta components of the catecholamines. Alpha agonist action has a negative effect on cyclic nucleotide levels. This effect is reduced by phentolamine, an alpha receptor antagonist, thus allowing inherent beta activity to be exerted. Blockade of the beta agonist action, on the other hand, removes its stimulatory effect and the negative alpha receptor activity is unopposed. In addition to the internal antagonism between the alpha and beta components observed in the action of epinephrine or norepinephrine, alpha agonist activity also suppressed cyclic AMP levels accumulated in response to adenosine.

Effect of methoxamine on isoproterenol- and adenosine-mediated increase in cyclic AMP. In view of the foregoing, it should be possible to reduce cyclic AMP levels by use of a pure alpha receptor agonist. Thus, we examined the effects of methoxamine on the stimulatory action of isoproterenol and adenosine. It was found (Table 2) that 0.1 mM methoxamine significantly reduced both isoproterenol- and adenosine-stimulated cyclic AMP levels. This action of methoxamine was largely reversed by phentolamine. It is significant that methoxamine reduced cyclic AMP levels augmented by adenosine. These data, like those in

Table 3. Combined effects of submaximal doses of adenosine and isoproterenol on accumulation of cyclic AMP*

Agents	Concn (μM)	Cyclic AMP (pmoles/mg protein) Observed	Expected (if additive)
Adenosine	10	9.6 ± 0.60 (4)	
Isoproterenol	0.05	5.9 ± 0.77 (3)	
Adenosine	10		
Isoproterenol	0.05	13.2, 13.0	11.8
Isoproterenol	0.5	8.5 ± 0.46 (5)	
Adenosine	10		
Isoproterenol	0.5	17.0, 17.7	14.4

* All incubations contained 0.1 mM Ro 20-1724. Observed values represent means ± S. E. M. from the number of experiments indicated in parentheses. Expected values were calculated from the increase in cyclic AMP produced by Ro 20-1724, adenosine or isoproterenol. The weighted average of the effect of Ro 20-1724 (Table 1) is 3.7. In the presence of Ro 20-1724, the increment due to adenosine (10 μM) was 5.9 and increments due to isoproterenol at 0.05 and 0.5 μM were 2.2 and 4.8 respectively.

Table 1, suggest that alpha agonist action antagonizes the accumulation of cyclic AMP mediated by either adenosine or a beta agonist.

Relationship between adenosine and beta agonist-induced responses. It was pointed out earlier (Table 1) that the stimulatory effect of isoproterenol and adenosine together was approximately the sum of their individual actions. In those experiments, both agonists were tested at concentrations which gave a maximal response. We considered it possible that under such conditions cyclic AMP formation might be limited by availability of substrate. Consequently, the combined effects of these two agonists were examined at concentrations (10 μM adenosine, 0.05 and 0.5 μM isoproterenol) which individually produced submaximal responses. The data in Table 3 indicate that, in the presence of Ro 20-1724, isoproterenol and adenosine together produced an effect on cyclic AMP formation which was essentially additve.

DISCUSSION

Increase in myocardial cyclic AMP in response to epinephrine has been shown to be due to stimulation of beta receptors which are considered to be coupled to adenylate cyclase. This catecholamine is known to activate both alpha and beta receptors. In brain slice preparations, it seems that stimulation of either alpha or beta receptors may lead to accumulation of cyclic AMP [9–12]; evidence is available that in other tissues [13–16] stimulation of alpha receptors may cause a decrease in the cyclic nucleotide. From the present studies, it appears that both alpha and beta receptors influence cyclic AMP formation, but in opposite directions. Alpha receptor stimulation is antagonistic to the positive effect of beta receptor input. Thus, the effect of isoproterenol was completely blocked by propranolol but was unaffected by phentolamine. Propranolol reduced epinephrine-stimulated levels to values lower than the control, while phentolamine enhanced epinephrine stimulation. The response to norepinephrine in the absence and presence of the blocking agents was similar to that produced by epinephrine, but smaller in magnitude. These data provide evidence that epinephrine and norepinephrine interact with both alpha and beta receptors causing a concomitant increase (beta effect) and decrease (alpha effect) on cyclic AMP formation. The observed stimulatory action of epinephrine is the balance between the two opposing effects with the beta component as the predominant one. In the case of norepinephrine, there appears to be only a weak beta stimulatory component and a large alpha inhibitory component. Thus, at 0.01 mM, norepinephrine appeared to have no stimulatory action on cyclic AMP levels, although a small but significant beta component could be revealed when the negative alpha agonist activity was blocked by phentolamine. More direct evidence supporting the opposing effects of alpha and beta agonist action is provided by the observation that methoxamine, a pure alpha agonist, reduced cyclic AMP stimulation by isoproterenol. This effect was partially reversed by phentolamine.

A negative effect of alpha receptor stimulation on cyclic AMP levels elevated through interaction with adenosine is also apparent from those experiments in which epinephrine or norepinephrine was added together with the nucleoside. Thus, phentolamine (which would block alpha receptors) markedly increased the action of the combined agonists, whereas propranolol (which would block beta receptors) depressed cyclic AMP levels well below those in the absence of the blocking agent, seemingly due to the now unopposed negative effect of alpha adrenergic input. The observations that methoxamine, a pure alpha receptor agonist, significantly suppressed adenosine-stimulated cyclic AMP levels, and that such action was largely reversed by phentolamine, provided additional evidence for suppressive action on cyclic AMP by alpha agonist activity.

While the stimulatory effects of adenosine and isoproterenol on cyclic AMP levels were essentially additive, the action of adenosine was significantly reduced in the presence of epinephrine or norepinephrine. It appears that the negative action of the alpha adrenergic component of either catecholamine is more pronounced when the cyclic AMP level in the tissue is elevated by adenosine stimulation. Whether there is direct antagonism between the actions of an alpha agonist and the nucleoside on adenylate cyclase cannot be assessed in the present study. It is also possible that alpha agonist activity could have suppressive action on cyclic AMP levels in general irrespective of how the levels are elevated. In the presence of Ro 20-1724, propranolol reduced epinephrine- or norepinephrine-stimulated levels to values lower than the appropriate control. This would suggest that the increase in cyclic AMP induced by inhibition of phosphodiesterase is also subject to antagonism by alpha agonist action.

The functional significance of alpha and beta adrenergic receptors in the heart has been intensively studied since the introduction of this classification by Ahlquist in 1948 [17]. A large body of evidence has accumulated which indicates that the positive inotropic action of catecholamines results from interaction with beta receptors and formation of cyclic AMP [18]. Positive inotropic effects evoked by stimulation of alpha adrenergic receptors has also been documented [19, 20] and evidence suggests that cyclic AMP is not involved in such processes [21, 22]. At this time little can be said about the possible functional significance of the negative effect of alpha receptor stimulation on cyclic AMP formation.

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