Br. J. Pharmac. (1974), 52, 97-100

MECHANISM OF CATECHOLAMINE ANTAGONISM IN RAT HEART PRODUCED BY PILOCARPINE AND RELATED DRUGS

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1 High concentrations of pilocarpine and methacholine consistently lowered the potencies of a series of adrenoceptor agonists as shown by displacement of complete cumulative dose-effect curves for their positive chronotropic action on rat isolated atria. The order of potency of the agonists was characteristic of β-adrenoceptor activation and this was converted to the type which characterizes α-adrenoceptor activation when pilocarpine was present.

2 Propranolol effectively blocked the adrenoceptor agonists in the presence of pilocarpine and phentolamine abolished the antagonistic actions of pilocarpine. Atropine, which by itself did not affect the action of the adrenoceptor agonists, abolished both the bradycardia and antagonism produced by pilocarpine.

3 It is concluded that pilocarpine antagonizes adrenoceptor agonists by muscarinic cholinoceptor activation without involving classical adrenoceptors.

Introduction

Recently we found that pilocarpine could antagonize the positive chronotropic effect of isoprenaline in the spontaneously beating, isolated, right atrium of the rat (Sadavongvivad & Satayavivad, 1974). The antagonism was not of the simple competitive type and was not proportional to the negative chronotropic effect of pilocarpine. The question arises whether the antagonism by pilocarpine has any relation to β-adrenoceptor blockade by known drugs and whether noradrenaline and other sympathomimetic amines are similarly antagonized.

This study deals with the results of a study of the mechanism by which pilocarpine antagonizes the actions of catecholamines on the heart.

Methods

The spontaneously beating, isolated, right atria of the rat were used in this study (Sadavongvivad & Satayavivad, 1974). The drugs used were: (−)-adrenaline bitartrate, (−)-noradrenaline hydrochloride, and (±)-propranolol hydrochloride from Sigma Chemical Co.; phentolamine hydrochloride from Ciba Pharmaceutical Co.; phenylephrine hydrochloride from Mann Research Lab.

Results

The influence of various concentrations of pilocarpine on the log dose-effect curves of noradrenaline, adrenaline and phenylephrine are summarized in Table 1.

Noradrenaline and pilocarpine interaction

The log dose-effect curve of noradrenaline was not modified by concentrations of pilocarpine that did not produce a negative chronotropic effect (10^{-7} M and below). When the effect of noradrenaline, measured in the presence of concentrations of pilocarpine greater than 10^{-7} M, was expressed as a percentage of the maximal response determined experimentally for each concentration of pilocarpine the log dose-effect curve of noradrenaline was shifted to the right as the concentration of pilocarpine was increased. Pilocarpine 10^{-3} M displaced the curve by 2 log units and this is qualitatively similar but quantitatively different from that found with isoprenaline (Sadavongvivad & Satayavivad, 1974 and Table 2). The maximal increase in heart rate due to noradrenaline increased with high concentrations of pilocarpine; this was associated with a reduction in heart rate and with an increase in the slope of the dose-effect curve (Table 1).

The influence of cocaine on noradrenaline and pilocarpine interaction

A family of log dose-effect curves of noradrenaline were measured in the presence of 10^{-5} M cocaine. Cocaine did not change the control noradrenaline

Table 1 Influences of pilocarpine on the relationship between log dose and positive chronotropic effect of various β-adrenoceptor agonists in the heart

Condition	Slope between 16-84% max mean	Slope between 16-84% max 95% confidence limit	pD_2 mean	pD_2 95% confidence limit	Δ max mean	Δ max 95% confidence limit
NA control	29.4	21.6-37.2	8.0	7.2-8.9	142	131-153
+Pi 10^{-6} M	32.1	17.7-46.6	7.7	6.2-9.2	119	84-154
+Pi 10^{-5} M	27.5	21.1-35.9	6.8	2.5-10.8	150	134-166
+Pi 10^{-4} M	46.9*	18.3-75.6	5.9*	4.6-7.4	186*	157-215
+Pi 10^{-3} M	37.4*	26.4-48.8	6.3*	5.3-7.3	183*	159-207
NA control	21.7	21.2-22.2	8.1	7.9-8.4	135	122-148
NA + Cocaine 10^{-5} M	23.2	16.7-29.6	8.5	7.0-9.9	136	123-149
+Pi 10^{-6} M	26.2	21.8-30.5	8.1	7.3-9.0	117	102-132
+Pi 10^{-5} M	22.3	15.3-29.4	7.5	5.6-9.3	123	107-139
+Pi 10^{-4} M	35.4*	14.3-56.5	6.3*	3.8-8.3	136	102-170
+Pi 10^{-3} M	41.4*	16.5-66.4	6.6*	4.7-8.5	164	139-189
AD control	16.8	15.1-18.5	8.8	8.1-9.5	138	127-149
+Pi 10^{-6} M	17.3	14.4-20.2	8.5	7.5-9.6	150	138-162
+Pi 10^{-5} M	16.9	14.4-19.4	8.2	7.3-9.2	137	114-160
+Pi 10^{-4} M	17.9	16.7-34.1	7.6	7.3-8.0	170*	150-190
+Pi 10^{-3} M	23.2*	12.3-34.1	6.9*	4.3-9.7	179*	155-203
PE control	36.1	25.6-50.3	5.6	5.1-6.8	113	100-126
+Pi 10^{-6} M	28.8	9.5-48.0	5.2	2.9-7.3	110	95-125
+Pi 10^{-5} M	35.1	21.0-49.2	5.7	4.4-7.1	106	91-121
+Pi 10^{-4} M	37.3	16.4-58.2	5.7	3.8-7.5	120	93-147

Effect expressed as percentage of maximum for each specified condition. NA, noradrenaline; AD, adrenaline; PE, phenylephrine; Pi, pilocarpine.
* Denotes significant difference from control.

dose-effect curve. The displacement and increased slope of the noradrenaline curves produced by high concentrations of pilocarpine were also not affected by cocaine. However, the increased height of these noradrenaline curves was abolished by cocaine; that is, the maximum heart rate which noradrenaline could produce was reduced.

Interaction between pilocarpine and other adrenoceptor agonists

The log dose-effect curve of adrenaline was shifted to the right by pilocarpine in a parallel manner except that pilocarpine 10^{-3} M caused a significant increase in the slope. The corresponding shift at each concentration of pilocarpine was slightly less for adrenaline than for noradrenaline.

The interaction of pilocarpine with phenylephrine was quite different from that found with the three catecholamines. The pD_2, slope and height of the dose-effect curve of phenylephrine were not altered by concentrations of pilocarpine up to 10^{-4} M. Plotted on an actual heart rate scale there was only a downward shift due to the change in base-line.

The results of experiments with various antagonists are summarized in Table 2 together with the results of an experiment in which methacholine was used instead of pilocarpine.

Influence of atropine on pilocarpine-isoprenaline interaction

In our previous report (Sadavongvivad & Satayavivad, 1974), pilocarpine 10^{-5} M reduced the pD_2 of isoprenaline from 11 to 3.3, increased the slope to 2.5 times and increased the range by 50%. In the present study atropine 10^{-5} M abolished completely these effects of pilocarpine. This concentration of atropine did not alter the log dose-effect curve of isoprenaline but blocked the negative chronotropic effect of pilocarpine completely.

Interaction between isoprenaline and methacholine

The influence of methacholine on the isoprenaline effect was similar to that of pilocarpine. The reduction in the pD_2 of isoprenaline and the

Table 2 Modification of the positive chronotropic effect of isoprenaline (ISO) by pilocarpine (Pi) and methacholine (Mc) and the influences of atropine (At), phentolamine (Ph), and propranolol (Pr)

Condition	Slope between 16-84% max mean	Slope between 16-84% max 95% confidence limit	pD_2 mean	pD_2 95% confidence limit	Δ max mean	Δ max 95% confidence limit
Iso control	19.9	16.9-22.9	9.8	8.9-10.7	152	135-169
Mc 10^{-6} M	30.6*	10.2-50.9	8.4	5.4-11.4	191*	172-210
Mc 10^{-5} M	38.1*	14.3-61.9	3.8*	1.9- 5.9	334*	284-384
Iso control	20.5	11.6-29.3	9.9	7.4-12.3	140	122-158
+Atr 10^{-5} M	20.3	11.3-29.2	9.7	7.5-11.9	143	124-162
+Atr 10^{-5} M +Pi 10^{-5} M	17.7	13.2-22.2	10.4	8.2-10.9	139	114-164
Iso control	18.4	16.2-30.5	10.5	8.7-12.6	159	142-176
+Pi 10^{-5} M	40.7*	18.1-56.7	4.5*	2.7- 6.7	200*	165-235
+Ph 10^{-5} M	17.3	14.1-20.8	10.0	7.2-11.9	161	142-180
+Pi 10^{-5} M +Ph 10^{-5} M	20.6	15.6-25.3	9.4	7.1-12.1	149	129-169
Iso control	21.5	14.7-22.7	9.5	8.0-13.6	154	139-169
+Pi 10^{-5} M	38.7*	18.8-60.9	4.2*,**	3.3- 5.0	197*	177-217
+Pr 10^{-6} M	20.1	15.3-25.6	6.1*	4.8- 7.6	160	140-180
+Pr 10^{-5} M	22.5	15.1-24.4	4.8*	3.0- 5.5	147	115-179
+Pi 10^{-5} M +Pr 10^{-6} M	40.2*	15.3-70.7	3.3*,**	2.1- 4.9	153	116-190
+Pi 10^{-5} M +Pr 10^{-5} M	36.5*	10.7-62.3	2.6*,**	1.7- 4.2	142	103-181

Effect expressed as percentage of maximum for each specified condition.
* Denotes significant difference from control.
** Denotes significant difference from that labelled ** in the same column.

increase in slope and height of the dose-effect curve produced by methacholine were comparable to those produced by pilocarpine at the same molar concentration.

Effects of adrenoceptor antagonists on the pilocarpine-isoprenaline interaction

In two separate experiments the effect of propranolol and phentolamine on the effect of isoprenaline were studied before and after pilocarpine 10^{-5} M. Repeated determinations of the isoprenaline dose-effect curve in the absence and presence of pilocarpine 10^{-5} M gave consistent results. Phentolamine at concentrations up to 10^{-5} M failed to influence the control isoprenaline curve significantly. The bradycardia caused by pilocarpine was also insensitive to phentolamine. However, the inhibitory effect of pilocarpine on isoprenaline was abolished almost completely by phentolamine. In the presence of both drugs at 10^{-5} M the isoprenaline curve was parallel and about one log unit to the right of the control curve. The maximal effects of isoprenaline in both cases were the same.

Propranolol remained active in blocking the effect of isoprenaline in the presence of pilocarpine. The increase in the height of the dose-effect curve seen in the presence of pilocarpine was blocked by propranolol. The bradycardia due to pilocarpine was not affected Hence, the shifts in the isoprenaline curve produced by both drugs were additive but the combined shift was less than the algebraic sum of the separate shifts.

Discussion

In rat isolated atria, pilocarpine produced two effects: bradycardia and antagonism of adrenoceptor activation. Since atropine blocked both the bradycardia and the catecholamine antagonism it was concluded that both these effects of pilocarpine were due to muscarinic cholinoceptor activation. We have obtained similar results with methacholine and this agrees with what other investigators (Carrier & Bishop, 1972; Grodner, Lahrtz, Pool & Brunwald, 1970) have found with acetylcholine; so it is likely that all muscarinic cholinoceptor agonists will produce similar effects.

The relative potency among the adrenoceptor agonists in our study in decreasing order are: isoprenaline, adrenaline, noradrenaline and phenylephrine. This order of potency fits the characteristics of β-adrenoceptor activation (Furchgott, 1967). The magnitudes of the decrease in potency due to pilocarpine at any effective

concentration are in the same order and, therefore, the intensity of pilocarpine antagonism varies according to the potency of the β-adrenoceptor agonist. However the positive correlation between the reduction in pD_2 and the potency as β-agonist is not because pilocarpine brings the potency of all the agonists to the same level so that the more potent one is shifted more than the others. Rather, the relative potency of each agonist remains different in the presence of the same concentration of pilocarpine. Thus, when the concentration of pilocarpine is 10^{-5} M or higher, the order of potency becomes: adrenaline, noradrenaline, phenylephrine and isoprenaline. This order of potency is the one that characterizes α-adrenoceptor activation (Furchgott, 1967). However, in the presence of pilocarpine, propranolol remains effective in blocking the effect of isoprenaline while phentolamine reverses the pilocarpine block. It seems, therefore, that we have here a novel type of adrenoceptor blockade that cannot be fitted into current adrenoceptor theory.

Based on the operational receptor concept proposed by Moran (1966) there may be two multistep sequential reactions which can be activated by two different operational receptors leading to opposite changes in heart rate. Our findings may be an example of the theoretical possibility that an antagonist may act outside a sequence of reaction and influence the sequence indirectly (Moran, 1967). Imagine that all adrenoceptor agonists produce tachycardia by activating a common sequence of reactions and that the cholinoceptor agonists activate another sequence which branches into the adrenoceptor-activated sequence to block it. If this cholinoceptor activated sequence were the one leading to bradycardia, the site where phentolamine blocks must be in the branched sequence.

According to this hypothesis, it is possible that a drug may be found which can activate the branched inhibitory sequence at a site after the cholinoceptor; this would be a new type of adrenoceptor antagonism and would be quite different from the known antagonists. Methoxamine and other α-adrenoceptor agonists, which James, Bear, Lang & Green (1968) reported as producing bradycardia when injected into the artery supplying the sino-atrial node of the dog, may be acting by this mechanism. On the basis that the bradycardia produced by these drugs can be blocked by phentolamine, these authors concluded that α-adrenoceptors exist in the heart. However, it is possible that these drugs may activate inhibition of the β-adrenoceptor pathway at the site where phentolamine has a blocking action.

We thank the Rockefeller Foundation for financial support toward equipment and materials. J. Satayavivad and P. Sanvarinda are predoctoral students in the Department of Pharmacology, Faculty of Science, Mahidol University.

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(Received January 18, 1974)