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ANTAGONISM OF THE EFFECT OF ISOPRENALINE ON HEART RATE BY PILOCARPINE

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1 The interaction of pilocarpine and isoprenaline on heart rate was investigated in the spontaneously beating, right isolated atrium of the rat.

2 The log dose-effect curve of isoprenaline, measured in the presence of various concentrations of pilocarpine, was not modified when the concentration of the latter did not exceed 10^{-6} M even though significant slowing of the heart had occurred. At concentrations higher than 10^{-6} M a rapid but graded shift to the right of isoprenaline response curves occurred so that at pilocarpine 10^{-5} M the pD_2 of isoprenaline was reduced from 11 to 3.3; cocaine slightly reduced this antagonism.

3 It is concluded that pilocarpine antagonizes isoprenaline by some mechanism which is not of the simple competitive type.

Introduction

Levy (1971) used the phrase 'accentuated antagonism' to refer to the finding, first reported by Samaan (1935), that the cardio-inhibitory nerve has a more powerful influence than the cardio-accelerator nerve upon heart rate. Current knowledge of the autonomic control of heart rate indicates that the predominance of the vagal influence on heart rate could be the result of various types of interaction at levels ranging from anatomical distribution of autonomic nerves to the subcellular level. Studies with isolated tissue indicate that this predominance remains at the tissue level and that acetylcholine can antagonize the positive chronotropic and inotropic actions of noradrenaline (Grodner, Lahrtz, Pool & Brunwald, 1970; Carrier & Bishop, 1972).

The purpose of this investigation was to characterize further the nature of combined cholinoceptor and adrenoceptor activation on heart rate using rat isolated atria. Isoprenaline was chosen rather than noradrenaline because the uptake of isoprenaline into the rat heart is insignificant (Hertting, 1964). Pilocarpine was used because it is more stable than acetylcholine.

Methods

Spontaneously beating right atria were removed from albino rats of either sex weighing 160-200 g and set up for measurement of isometric force of contraction by the method described previously (Kennedy, Nookhwun, Sadavongvivad & Tanchajja, 1971). Heart rate was measured by a cardiotachometer (Sanborn 350-3400A). Tissues were left for 1 h in the organ bath at 35° C before the effects of drugs were studied. All drugs ((±)-isoprenaline hydrochloride, pilocarpine nitrate, Sigma Chemical Co.; cocaine hydrochloride, E. Merck, Germany) were dissolved in ascorbic acid solution (10^{-4} M) in such concentration that the desired bath concentration could be achieved without changing the volume of the bath fluid by more than 1%.

Cumulative dose-effect relationships were determined in all experiments. The maximum effect (used for calculating the percentage of maximum effect) was determined at the beginning of each experiment by adding isoprenaline 10^{-6} M to the bath; the average of three such determinations was taken as the maximal effect obtainable from that atrium. Higher concentrations of isoprenaline rarely caused a further increase in heart rate but always caused a lower force of contraction than at 10^{-6} M. Where there was a change in the potency of isoprenaline, the maximum effect was determined by continuing to add the drug until a 100-fold increase in concentration did not increase the effect further.

Dose-effect curves of isoprenaline were determined first in the absence of pilocarpine and then in various concentrations of pilocarpine from 10^{-12} M to 10^{-4} M. The average slope of the log dose-effect curve between 16% and 84% of the maximum effect was determined by linear regression. Four points were used for each

regression with each point containing 15-20 measurements in different preparations. The pD_2, the negative logarithm of the molar concentration producing a half-maximum response, was calculated from the regression equation. Linearity of the curves and comparison of slopes were performed by analysis of variance. All statistical analyses were performed according to Sokal & Rohlf (1969).

Results

Influence of pilocarpine on the positive chronotropic effect of isoprenaline

Table 1 summarizes the influence of pilocarpine on the parameters of the curve relating log dose of isoprenaline to the positive chrontropic effect, expressed as percentage maximum. At concentrations of 10^{-7} M and below pilocarpine did not produce a significant negative chronotropic effect and the isoprenaline curve was not different from the control curve. Pilocarpine 10^{-6}, 10^{-5} and 10^{-4} M reduced the resting heart rate by 10-20%, 30-40% and 50-70% respectively but the associated changes in the isoprenaline dose-effect curve were clearly not linearly related to the negative chronotropic actions. Pilocarpine 10^{-6} M caused a 10-fold reduction in the potency of isoprenaline but this was not statistically significant. However, pilocarpine 10^{-5} M reduced the pD_2 of isoprenaline by 7.7 log units representing some 10 million-fold decrease in potency. Other experiments with concentrations of pilocarpine between 10^{-6} and 10^{-5} M indicated that this shift is graded.

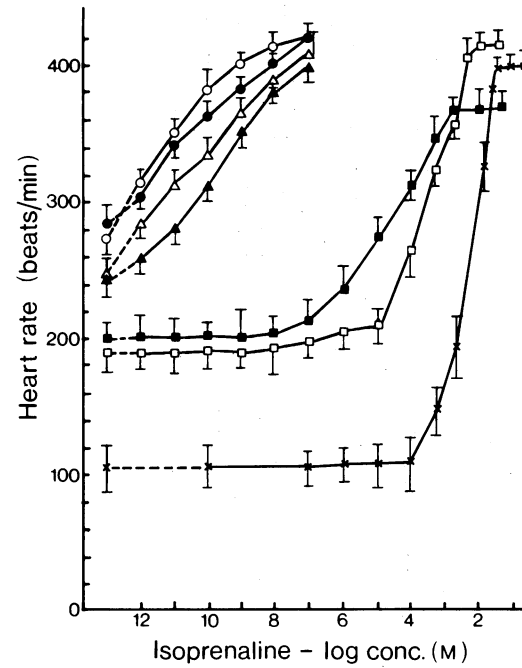

Fig. 1 Influence of pilocarpine on the positive chronotropic effect of isoprenaline and the effects of cocaine. Open symbols, no cocaine present. Solid symbols, cocaine 10^{-5} M present. Each curve is the effect of isoprenaline determined in the presence of pilocarpine (○, ●) 0 M; (△, ▲) 10^{-6} M; (□, ■) 10^{-5} M; (x) 10^{-4} M. Each point represents the mean with s.e. mean of 15-20 measurements. Data taken from the same experiments as Table 1 but not all points used in the calculations of Table 1 are shown on the graph.

Table 1 Comparison of isoprenaline log dose-effect curves measured at various concentrations of pilocarpine (Pi) and the influence of cocaine.

Condition	*Slope between 16-84% max.*		*pD_2*		*Δ Max. measured*
	Estimated	*95% Confidence limit*	*Estimated*	*95% Confidence limit*	*95% Confidence limit*
Control	18.5	6.1-31.0	11.0	8.0-14.1	149 ± 15
+Pi 10^{-6} M	13.6	6.8-20.4	10.0	7.7-12.2	158 ± 15
+Pi 10^{-5} M	45.8*,**	16.2-77.0	3.3*,**	2.6-4.6	224 ± 51*,**
+Pi 10^{-4} M	146.6*	47.0-242	2.5*	1.5-3.4	296 ± 68*
+Cocaine 10^{-5} M	21.8	15.1-28.6	10.8	9.5-12.2	120 ± 16
+Pi 10^{-6} M	23.5	16.9-30.1	10.2	9.0-11.4	134 ± 16
+Pi 10^{-5} M	31.3**	24.2-38.4	4.3*,**	3.7-4.9	165 ± 10**

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

Increase in pilocarpine concentration to 10^{-4} M caused a further small decrease in pD_2 of isoprenaline. The isoprenaline dose-effect curves were shifted without any decrease in the maximal heart rate that it produced (Fig. 1); since pilocarpine decreased the resting heart rate the maximum response and the slope of the dose-effect curves were therefore increased. The latter remained prominent when the responses were expressed as fractions of their respective maxima (Table 1).

Effects of cocaine

Since it has been suggested that pilocarpine might promote the uptake of isoprenaline into adrenergic nerve endings (Hamilton, 1971), the possibility that cocaine might modify this effect of pilocarpine has been examined. Figure 1 and Table 1 show that cocaine did not alter the negative chronotropic effect of pilocarpine itself. However, cocaine reduced the decrease in potency of isoprenaline produced by pilocarpine and also reduced the maximum heart rate which isoprenaline could produce in the presence of pilocarpine 10^{-5} M.

Discussion

Functional antagonism occurs when two drugs interact independently with different receptor systems to produce opposite effects on a common effector system; an approximation to algebraic summation of effects is expected when drugs with these properties are combined (Ariens, van Rossum & Simonis, 1957). The effects of acetylcholine and noradrenaline released from vagus and sympathetic nerves respectively, might be expected to combine in this way in affecting heart rate. However, recent investigators (Levy & Zieske, 1969; Grodner *et al.*, 1970; Carrier & Bishop, 1972) studying the combined influence of the two divisions of the autonomic nervous system on heart rate agree with Rosenblueth & Simeone (1934) that their combined influence is not the algebraic sum of the separate effects. The conclusion of Rosenblueth & Simeone that the two nerves act independently was rejected in favour of a complex interaction (Levy, 1971) derived from the findings of all investigators since Samaan (1935).

Algebraic summation of the combined effect implies that if the data from experiments reported here are plotted as actual heart rate as in Fig. 1 there will be a vertical downward shift of the isoprenaline curve without any change in the slope, pD_2 or maximum increase. Theoretically, the predominance of the cholinoceptor agonist may be shown by decrease in the maximal heart rate obtainable by the adrenoceptor agonist with or without simultaneous change in the slope of the shifted curve. Decrease in both the absolute maximum and slope is implicated in the finding of Rosenblueth & Simeone (1934). From our results reported here, the negative chronotropic effect of pilocarpine has greater influence than the positive chronotropic effect of isoprenaline mainly because there is a reduction in the potency of isoprenaline. It is conceivable that algebraic summation may also exist but is masked by the change in potency. That this is unlikely is evident from the fact that the slope and maximum also increase. The reduction in the potency of isoprenaline in the presence of pilocarpine clearly indicates that pilocarpine can antagonize isoprenaline. However, the non-parallel shift and the relation of the displacement to pilocarpine concentration indicates that this is not simple competitive antagonism.

In the whole animal, pilocarpine and other cholinoceptor agonists can convert the depressor effect of isoprenaline to a pressor one (Daniell & Bagwell, 1969; Hamilton, 1971). Hamilton found that cocaine can abolish this effect of pilocarpine on the depressor effect of isoprenaline and suggested that pilocarpine acts on adrenergic nerve endings to permit uptake of isoprenaline which then acts like an indirectly-acting sympathomimetic amine. The results with cocaine reported here show only a slight reduction of pilocarpine antagonism. As far as it goes, this fits the cocaine-sensitive, pilocarpine-induced uptake of isoprenaline postulate. However, our findings do not fit the assumption that isoprenaline is acting like an indirectly-acting sympathomimetic amine in the presence of pilocarpine. In that case cocaine, by blocking the uptake of isoprenaline and consequently the release of noradrenaline, should increase the antagonism of pilocarpine.

The nature of the antagonism between pilocarpine and isoprenaline remains to be clarified.

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