J. Pharm. Pharmac., 1973, **25**, 55-59 *Received July* 7, 1972

The adrenoceptors mediating catecholamine effects in frog isolated skin

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The short circuit current (s.c.c.), potential difference (p.d.) and resistance of the frog skin have been studied. Sympathomimetics increased the s.c.c. in order of relative potency: isoprenaline > salbutamol > noradrenaline. The sympathomimetic-induced increase in s.c.c. was antagonized by propranolol and not by phentolamine. A large concentration of practolol was needed to antagonize the isoprenaline-induced increase in s.c.c. The antidiuretic hormone (ADH)-induced increase in s.c.c. was unaffected by propranolol, practolol or phentolamine. The results suggest that the sympathomimetic-induced increase in s.c.c. is mediated by β_2-adrenoceptors.

It is well known that catecholamines increase the short circuit current (s.c.c.) in frog isolated skin (Koefoed-Johnsen, Ussing & Zerahn, 1953; Watlington, 1968; House, 1969; Lindley, 1969). Fassina, Carpenedo & Fiandini (1968) compared the activity of catecholamines on the preparation by determining their concentration-effect curves. They showed that the order of relative potency was isoprenaline > noradrenaline > adrenaline. This, together with sensitivity to the β-adrenoceptor blocking agent Kö 592 (1-(2-methylphenoxy)-3-isopropylamino-2-propanol), suggested that the increase in s.c.c. of the frog skin was mediated by β-adrenoceptors.

We have attempted to find whether the adrenoceptor mediating increase in s.c.c. in frog skin can be placed with one or other sub-groups of mammalian β-adrenoceptors suggested by Lands, Arnold & others (1967).

METHODS

The method of Ussing & Zerahn (1951) was used. Frogs (*Rana temporaria* 25 to 40 g) were pithed and the ventral skin was dissected free from adhering tissues. The skin was placed between two Perspex chambers so that it separated two identical Ringer solutions, each of volume 40 ml.

Experimental procedure

The Ringer solutions bathing the serosal and mucosal surfaces of the frog skin were changed twice during the early part of an equilibration period of 2 h. The resting p.d. and the s.c.c. were measured once every 5 min. Between readings which were made under absolutely standardized conditions the skin was kept open circuited. Stability of the measured values were taken to indicate equilibration. Cumulative concentration effect curves were obtained for drugs that were added to the serosal chamber. Readings of p.d. and s.c.c. were again taken at 5 min intervals. Each concentration increment was allowed a 15 min contact time, to give full development of its effect.

In some experiments, after a single concentration effect curve had been obtained, the mucosal chamber was washed with normal Ringer solution and the serosal

chamber was washed with Ringer solution containing an antagonist. The skin was equilibrated again for 2 h before new values of the p.d. and s.c.c. equal to or less than the original values were obtained. Then an agonist cumulative concentration effect curve was repeated.

The increase in s.c.c. produced by each concentration of an agonist was calculated and expressed as a proportion (%) of the maximum increase in s.c.c. produced by the agonist in normal Ringer solution.

RESULTS

The effects of sympathomimetics

(±)-Isoprenaline, salbutamol and (−)-noradrenaline each caused a concentration-dependent increase in the s.c.c. (Fig. 1). At the EC 50 level noradrenaline was

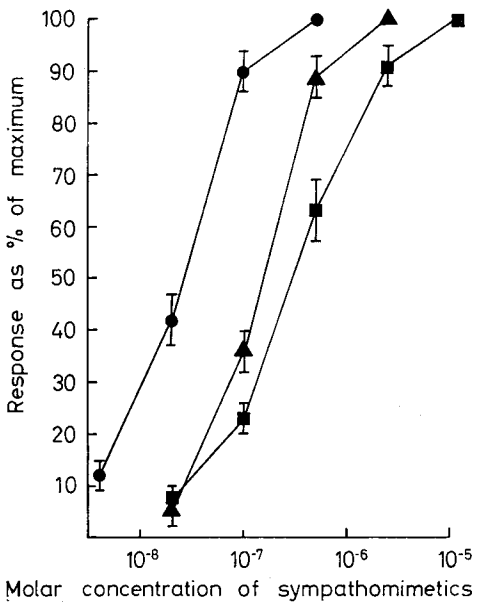

FIG. 1. Log concentration effect lines of isoprenaline (●—●), salbutamol (▲—▲) and noradrenaline (■—■). Increase in s.c.c. measured as % of maximum was plotted against molar concentration of sympathomimetic on a log scale. Each point is the mean of 7 experiments ± s.e. Mean short circuit current values (μA) before drug addition and mean maximum increase in these values (μA) were respectively: isoprenaline 91,121; salbutamol 85,65; noradrenaline 99,123.

11·2 times (log concentration ratio 1·05 ± 0·22) and salbutamol 5·8 times (log concentration ratio 0·76 ± 0·19) less potent than isoprenaline. These drugs also increased the p.d. and decreased the skin resistance in a concentration-dependent fashion.

Neither phenylephrine (64 μM) nor xylometazoline (100 μM) reduced the s.c.c. or the increase in s.c.c. induced by ADH (10 mU/ml), or the increase in s.c.c. induced by isoprenaline (20 nM). Each effect was tested four times.

Experiments using antagonists

Propranolol (640 nM) and practolol (100 μM) each produced a similar parallel shift to the right of the log concentration effect curve for isoprenaline (Fig. 2A,B).

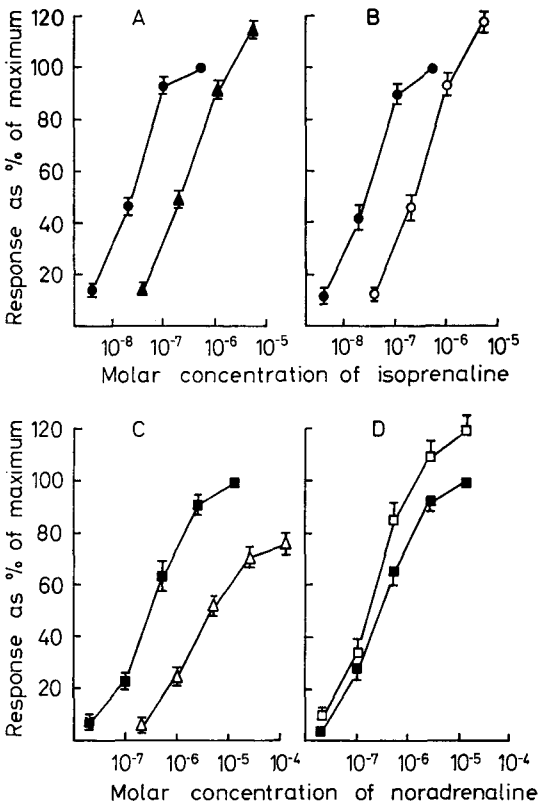

FIG. 2. Effects of propranolol, practolol and phentolamine on the increase in s.c.c. produced by catecholamines. Increase of s.c.c. measured as % of maximum increase produced by the agonist in normal Ringer solution, was plotted against molar concentration of agonist on a log scale. ●—● Isoprenaline in normal Ringer solution. ▲—▲ Isoprenaline in Ringer solution containing 640 nM propranolol. ○—○ Isoprenaline in Ringer solution containing 100 μM practolol. ■—■ Noradrenaline in normal Ringer solution. △—△ Noradrenaline in Ringer solution containing 640 nM propranolol. □—□ Noradrenaline in Ringer solution containing 3·2 μM phentolamine. Each point is the mean of 7 experiments ± s.e. Mean short circuit current values (μA) before drug addition and mean maximum increase in these values (μA) before addition of antagonist were respectively: A 89,128; B 95,112; C 101,126; D 95,116.

Propranolol (640 nM) also produced a shift of the log concentration effect curve for noradrenaline (Fig. 2C). Phentolamine (3·2 μM) produced a slight shift to the left of the log concentration effect curve for noradrenaline (Fig. 2D).

Propranolol (640 nM), practolol (100 μM) and phentolamine (3·2 μM) each had no effect on the s.c.c. or on the ADH (10 mU/ml)-induced increase in s.c.c. Each effect was tested four times.

Although a second isoprenaline log concentration effect curve showed the same EC 50 as the first, it always produced a greater maximum than the first. However, as seen in Fig. 2C, the maximum effect of noradrenaline in the presence of propranolol was smaller than before.

DISCUSSION

Lands & others (1967) further classified the concept of Ahlquist (1948) of two types of adrenoceptors (α and β) in effector tissues into two sub groups, β_1 and β_2, on the basis of numerical differences in the relative potency of agonists.

We found noradrenaline 11·2 times less potent than isoprenaline in increasing the s.c.c. of the frog skin, while Fassina & others (1968) found that noradrenaline was more potent than adrenaline. This order of potency is also found for adrenergic lipid mobilization in mammals (Barrett, 1965) which Lands & others (1967) attribute to activation of β_2-adrenoceptors.

We found salbutamol to be only 5·8 times less potent than isoprenaline in increasing the s.c.c. of the frog skin. Salbutamol is an agonist which is much more active on bronchial and vascular smooth muscle than on cardiac muscle (Cullum, Farmer & others, 1969; Farmer, Levy & Marshall, 1970) and on the basis of Lands & others (1967) classification it can be regarded as a selective agonist at β_2-adrenoceptors.

The large concentration of practolol (100 μM) we used was needed to antagonize the effect of isoprenaline, whereas a small concentration of propranolol (640 nM) was enough to bring about an equal parallel shift to the right of the log concentration effect curve for isoprenaline. Dunlop & Shanks (1968) showed practolol to be roughly four times less potent than propranolol as an antagonist of the cardiac actions of catecholamines but much the less effective in antagonizing the relaxations of vascular and bronchial smooth muscle. Thus on the basis of Lands & others (1967) classification practolol can be regarded as a selective antagonist at β_1-adrenoceptors.

These results indicate that the increase in s.c.c. of the frog isolated skin is mediated by adrenoceptors corresponding to the sub group β_2 of Lands & others (1967). However, it is to be noted that in dogs the vasodilator action of salbutamol is about 1/10 that of isoprenaline but the drugs are nearly equiactive on bronchial muscle (Cullum & others, 1969). Farmer & Levy (1970) found practolol to be more potent than expected on guinea-pig vas deferens and less potent on rabbit ileum. They agreed with the concept that there are different β-adrenoceptor mechanisms but warned that the whole body of results may be difficult to reconcile with the simple two receptors hypothesis of Lands & his colleagues.

Fassina & others (1968) reported that "autoinhibition", a reduction in the % increase in s.c.c., developed with higher doses of catecholamines and showed the following order of intensity: adrenaline > noradrenaline > isoprenaline. Watlington (1967, 1968) suggested the presence of α-and β-adrenoceptors in frog skin. He showed that adrenaline in the presence of pronethalol reduced the s.c.c. We have observed neither autoinhibition, unless the lower maximum response to noradrenaline in the presence of propranolol is a reflection of this phenomenon, nor a reduction in the s.c.c. with noradrenaline either before or after propranolol treatment.

Xylometazoline is a "specific directly acting α-sympathomimetic devoid of β-sympathomimetic actions" which is 6·4 times less potent than (—)-noradrenaline on the rabbit gut, 1·3 times less potent on the rat vas deferens and 100 times less potent on the cat blood pressure (Mujic & van Rossum, 1965). The failure of phenylephrine and xylometazoline to reduce the s.c.c. or the increase in s.c.c. induced by either ADH or isoprenaline in our experiments does not lend any support to the idea that there are α-adrenoceptors in frog skin. The concentrations of xylometazoline (100 μM) and phenylephrine (64 μM) we used would have high activity at mammalian α-adrenoceptors. Although α-adrenoceptors in the amphibia may differ slightly from these, phenylephrine does have significant α-adrenoceptor agonist activity on the toad bladder (Ambalavanar, Foster & Schnieden, 1972). The difference between our results and the results of Fassina & others (1968) and Watlington

(1967, 1968) may be due to differences of technique and species. Fassina & others used frogs of the species *Rana esculenta* and also short circuited the skin between readings while Watlington used *Rana pipiens.*

Acknowledgements

We wish to thank Dr. J. D. Fitzgerald of Imperial Chemical Industries Ltd., for the gifts of propranolol and practolol.

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