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Effects of sympathomimetics on water movement across toad isolated bladder

That antidiuretic hormone (ADH) increases water movement out of toad isolated bladder is well known. Some catecholamines inhibit this action of ADH (Handler, Bensinger & Orloff, 1968; Strauch & Langdon, 1969). Handler & others (1968) have suggested that this action of catecholamines is probably mediated by α-adrenoceptors. The basis of this suggestion was inhibition by adrenaline and noradrenaline but not by isoprenaline, and antagonism of this inhibition by phentolamine (10^{-4}M) and phenoxybenzamine (10^{-4}M) but not by propranolol (10^{-4}M). We have sought to confirm this conclusion and extend the observations in three directions—to show that the inhibitory action of directly-acting sympathomimetics is concentration dependent, to use a wider range of agonists, and to employ concentrations of antagonists likely to be more specific in their actions.

Methods described by Bentley (1958) and Handler & others (1968) were used. Dividing the bladder of *Bufo bufo* (25 to 40 g) into two half-bladders provided a concurrent paired control to every experiment. Pairing was effective in that the spontaneous water loss and the stimulation of water loss induced by ADH, 250 μU/ml (the experimentally determined ED 50), did not differ ($P \simeq 0{\cdot}7$) between the right and left half-bladder of 6 toads. We also checked that the water loss during a second 40 min period of observation did not differ from that during the first period (correlation coefficient 0·95, $P < 0{\cdot}01$).

(−)-Adrenaline (10^{-7}M) did not affect spontaneous water movement ($P \simeq 0{\cdot}55$). However it completely inhibited the water loss induced by ADH (250 μU/ml). (−)-Noradrenaline (8×10^{-8}M), (−)-phenylephrine (2×10^{-6}M), dopamine ($2{\cdot}5 \times 10^{-6}$M) and (±)-isoprenaline (5×10^{-4}M) also had no effect on the spontaneous water loss. All five sympathomimetics produced a concentration-dependent inhibition of water movement (Fig. 1.)

Phentolamine was studied at a concentration, $2{\cdot}8 \times 10^{-7}$M, which caused approximately ten-fold antagonism of sympathomimetics acting directly at the α-adrenoceptors of mammalian vascular smooth muscle (Ambalavanar, Foster, Kelly & Schnieden: unpublished observations). It did not affect either the spontaneous water loss from the toad bladder or the loss induced by ADH (250 μU/ml). Its effect on the activity of each of the five sympathomimetic amines was assessed using a null method. The mean water loss of six half-bladders treated with ADH (250

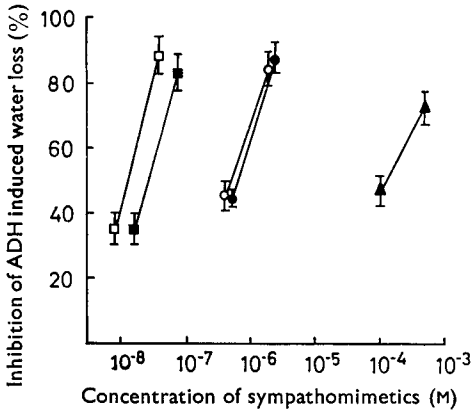

FIG. 1. Log concentration effect lines of sympathomimetics. % inhibition of ADH (250 μU/ml) induced water loss is plotted against concentration of sympathomimetics on a log scale. □Adrenaline, ■noradrenaline, ○phenylephrine, ●dopamine, ▲isoprenaline. Each point is the mean of 5 experiments ± s.e.

μU/ml) + ten times the EC 50 of sympathomimetic + phentolamine ($2{\cdot}8 \times 10^{-7}$M) was compared with that of the six paired control half-bladders treated with ADH (250 μU/ml) + the EC 50 of sympathomimetic. In each case there was no significant difference in water movement. Therefore this concentration of phentolamine caused approximately ten-fold antagonism of sympathomimetics acting on the toad bladder.

Propranolol was studied at a concentration, 8×10^{-7}M, which caused approximately ten-fold antagonism of sympathomimetics acting at the β-adrenoceptors of the frog skin (Ambalavanar, Foster & Schnieden, unpublished observations). It had no effect on the spontaneous water flow down the osmotic gradient, on the ADH-induced water loss or on the inhibition of this loss caused by any of the sympathomimetics.

The establishment of the relative potency and concentration dependence of the action of five agonists and the employment of smaller concentrations of antagonists consolidates the evidence for α-adrenoceptor mediation of catecholamine inhibition of ADH action on the toad bladder (Handler & others, 1968).

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