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The effect of temperature on the α-adrenoceptor antagonist potency of indoramin and labetalol in the rat perfused mesenteric vascular bed

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The effect of reduction in temperature of perfusion from 37 to 27 °C and 20 °C on the ability of the adrenoceptor antagonists indoramin and labetalol to block pressor responses to noradrenaline in the perfused mesenteric vascular bed of the rat was examined. The results suggest that in the rat mesenteric bed, antagonist potency of both agents is increased at low temperatures.

Temperature is a factor that changes the adrenergic neuroeffector interaction, and vascular smooth muscle responses can be profoundly altered by local changes in temperature (Vanhoutte 1980). It has previously been reported (Clement 1980) that the ability of indoramin (a selective α_1-adrenoceptor antagonist; Rhodes & Waterfall 1978) to increase blood flow in the cooled hands of acrocyanotic patients was less marked at low temperatures than at 37 °C. An investigation was therefore undertaken to examine the changes in α-adrenoceptor antagonist action of indoramin with temperature in an isolated vascular bed. A comparison was made with the mixed α and β-adrenoceptor antagonist labetalol, previously described by Brittain & Levy (1976).

Materials and methods

The perfused mesenteric vascular bed of the rat was prepared using a modification of the method described by McGregor (1965).

Male Sprague Dawley rats (180–220 g) were anaesthetized by intraperitoneal injection of 60 mg kg^{-1} of pentobarbitone sodium (May & Baker). The abdomen was opened by midline incision, the ileum and colon exposed and the superior mesenteric artery identified and cannulated. The perfusion pump was in operation during the cannulation process. The perfused area was identified by blanching and included an area of the ileum and caecum. The caecal, ileo-colic, colic and pancreatico-duodenal branches of the artery were tied off and the perfused mesentery was severed close to the ileum obviating possible interference from the activity of intestinal smooth muscle.

The perfused mesenteric bed was placed in a water-jacketed bath and maintained at either 37, 27 or 20 °C as required. Perfusion was set at 2 ml min^{-1} using a Watson Marlow flow inducer. Krebs-bicarbonate solution (mM): NaCl 118, KCl 4·8, $NaHCO_3$ 25·0, $MgSO_4$ 1·2, glucose 11·1, $CaCl_2$ 2·5, gassed with 5% CO_2 in O_2, and containing ascorbic acid (10^{-4} M) and 0·1% Bovine serum albumin factor V (to prevent drug oxidation and reduce tissue oedema respectively) was used as perfusate. The concentration of solutions of noradrenaline (Koch-Light) (NA) were such that the bolus volume of NA injected was within the range 5–50 μl. Pressor responses to bolus injections of NA were recorded via Bell and Howell type 4/422 pressure transducers, connected to a Devices MX4 polygraph.

Indoramin (Wyeth) was added to the perfusion fluid at concentrations of either 10^{-7} M (at 37 and 27 °C) or 10^{-9} M (at 20 °C) and allowed to equilibrate in the tissue for 30 min.

In separate preparations, labetalol (Glaxo, Ware) was added to the perfusion fluid at concentrations of 10^{-6} M (at 37 °C) and 3×10^{-8} M (at 27 and 20 °C) and equilibrated as before. Pressor responses to bolus injections of NA were again obtained in the presence of antagonist and dose-response curves to NA constructed before and after the addition of antagonist, from which antagonist pA_2 values were calculated using the method of Van Rossum (1963). Control tissues were also

equilibrated with antagonist-free Krebs solution (after an initial concentration-response curve to NA) at the appropriate temperature. Antagonists were tested on at least four tissues at each temperature, together with two additional antagonist-free controls to assess (by measurement of EC50 values for controls) changes in sensitivity to NA with time. Results are shown in Table 1.

Table 1. The effect of lowering temperature on pA_2 values (mean ± s.e.m.) for indoramin and labetalol against pressor responses to NA in the perfused mesenteric bed of the rat (n = 4–6).

	37 °C	27 °C	20 °C
Indoramin	8·6 ± 0·1	9·2 ± 0·2	10·0 ± 0·1
Labetalol	7·4 ± 0·1	8·0 ± 0·1	8·5 ± 0·2

Results

Reduction in temperature was associated with an increase in the α-adrenoceptor antagonist potency of indoramin. A similar apparent increase in potency was observed with labetalol. With both drugs, as temperature decreased, the time to reach maximum response in the presence of NA was prolonged, and 'washout time' (i.e. time taken to return to base-line perfusion pressure) increased.

Reducing the temperature from 37 to 27 °C increased the pA_2 of indoramin from 8·6 to 9·2 and that of labetalol from 7·4 to 8·0. Further reduction to 20 °C increased the pA_2 values to 10·0 and 8·5 respectively. The sensitivity of control tissues to NA did not vary with temperature or time during the course of the procedure.

Discussion

In the rat mesenteric bed at 37 °C indoramin has previously been reported to be a competitive antagonist of the pressor effects of NA (Collis & Alps 1973). The results of the present study show a marked increase in antagonist potency of indoramin with decreasing temperature. A similar relationship was observed with labetalol.

Vanhoutte et al (1981) have shown that, in the hind limb of the dog, a reduction in temperature augments the pA_2 value of the competitive α-adrenoceptor blocker phentolamine. The present findings with indoramin are in agreement with this study and suggest an increase in the affinity of the antagonist for the receptor site (Vanhoutte et al 1981), as temperature falls. The data also suggest that the results obtained by Clement (1980) may not be attributable to reduced α-adrenoceptor blockade by indoramin at low temperatures, although the effects at temperatures below 20 °C could not be readily determined on the mesenteric bed.

With labetalol, the pA_2 value obtained at 37 °C (7·4 ± 0·1) was similar to the α-adrenoceptor antagonist pA_2 against NA in both the rabbit and rat aortic strips (7·44 and 7·42 respectively) and the rat vas deferens (7·45) as described by Brittain & Levy (1976). Unpublished findings from this laboratory suggest that the β-adrenoceptor antagonist propranolol (10^{-6} M) produced no change in the dose-response relationship to NA in the perfused mesenteric bed. It is therefore assumed that in this preparation labetalol is exerting its effects mainly by antagonism of α-adrenoceptors.

In conclusion, the present study shows that, in the rat mesenteric bed, the α-adrenoceptor blocking potency of indoramin over the temperature range 37–20 °C is increased as temperature is reduced. A similar finding was observed with labetalol.

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