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Hypothermia induces supersensitivity of mouse vas deferens to adrenergic agonists: evidence for postjunctional α_2-adrenoceptors

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Abstract—Hypothermia-induced potentiation of α-adrenoceptor-mediated responses of the mouse vas deferens to noradrenaline is mainly caused by inhibition of the sites of loss. However, even after blockade of the sites of loss for noradrenaline or when using methoxamine (which is not a substrate for uptake or metabolism) hypothermia still causes a significant increase in responsiveness. This remaining supersensitivity was shown to be an increased receptor affinity. Furthermore, hypothermia revealed contractile responses to low concentrations of UK 14304 (an α_2-agonist).

It is well known that the response of smooth muscles to drugs varies with the temperature of the bathing medium. Reduction in temperatures enhances the contractile responses evoked by exogenous noradrenaline in various tissues; namely in mouse vas deferens (Buckner et al 1975), in rat vas deferens (French et al 1986) and in dog saphenous vein (McAdams & Waterfall 1984; Vanhoutte & Flavahan 1986). The hypothermia-induced potentiation of responses to noradrenaline was attributed to inhibition of the mechanisms that are responsible for the removal of the amine from the biophase (Buckner et al 1975; McAdams & Waterfall 1984; French et al 1986). In the dog saphenous vein (a tissue possessing both postjunctional α_1- and α_2-adrenoceptors), inhibition of these mechanisms may account for supersensitivity; however, even after blockade of the sites of loss, hypothermia still causes a significant increase in responsiveness to noradrenaline. This supersensitivity was ascribed to the increased responsiveness of α_2-adrenoceptors (Vanhoutte & Flavahan 1986). Moreover, in the same paper the authors reported that cooling depresses α_1-adrenergic responses. The purpose of this work was to study the effect of temperature on the sensitivity of the mouse vas deferens to different agonists and to examine how α-adrenoceptors contribute to the changes in responsiveness, in a tissue where α_1-adrenoceptors predominate. Preliminary results were presented to the 6th International Catecholamine Symposium, Jerusalem, 1987 (Albuquerque et al 1987) and to the 3rd Reunion Luso-Española de Farmacologia, 1987 (Gonçalves et al 1987).

Materials and methods

The vasa deferentia of freshly killed Charles River mice, 30–40 g, were removed and dissected free of surrounding tissue. The whole vas deferens was suspended in a tissue bath of 15 mL capacity in Krebs Henseleit solution gassed with carbogen (95% O_2+5% CO_2). The tissue was mounted under 0·2 g tension connected via an isotonic transducer (mod. Ugo Basile 7006) to a recorder (mod Ugo Basile 7050). Before the start of the experiment, preparations were equilibrated in Krebs solution for 40 min and washed every 10 min. Concentration-response curves were obtained by the single dose method and the doses were added to the bath at intervals of 10 min. Two concentration-response curves were obtained in each preparation for the agonist under study: noradrenaline (NA) or methoxamine. The first curve was obtained at 37°C and the second one at 37 (control), 20 or 12°C. 60 min were allowed to elapse between the end of the first concentration-response curve and the start of the second. Bath temperature was maintained at the selected temperature for 50 min before and during the second concentration-response curve. The change of temperature was complete within a 5 min period. When NA was used, the concentration response curves were obtained in the absence or in the presence of 12 μM cocaine (C), 60 μM hydrocortisone (H) and 1 μM propranolol (P) to block neuronal uptake, extraneuronal uptake and β-adrenoceptors, respectively. These drugs were added to the bath solution 30 min before the first addition of the agonist and remained there until the end of the experiment. Responses were expressed as percentage of the maximum response at 37°C and the EC_{50} values were calculated for the different temperatures. Responses to UK 14304 (a selective α_2 agonist) were obtained at various temperatures (37, 20 and 12°C) only in two different concentrations on each vas deferens. Dissociation constants (K_A) for NA were determined in the presence of C+H+P at 37 or 20°C by using phenoxybenzamine (5 nM) to partially inactivate α_1-adrenoceptors. K_A were calculated according to the method of Furchgott (1966). Antagonist dissociation constants (K_B) of prazosin (a selective α_1-antagonist) were determined (in the presence of C+H+P) from concentration response curves for NA at 37 or 20°C. Prazosin (5 nM) was added to the bath 30 min before the second response curve and maintained until the end of the experiment. The K_B was calculated according to Furchgott (1972). Geometric means and 95% confidence limits for the EC50, the K_A and the K_B were calculated. Differences between means were compared by Student's *t*-test and those with *P* values of 0·05 or less were considered significant.

Drugs. The drugs used were: cocaine hydrochloride (Uquipa), (−)-noradrenaline bitartrate, hydrocortisone phosphate and propranolol hydrochloride (Sigma), (±)-methoxamine hydrochloride (Wellcome), phenoxybenzamine hydrochloride (Smith Kline, French), prazosin hydrochloride and UK 14304 [5-bromo-6(2-imidaxolin-2-ylamine)-quinoxaline] (Pfizer).

Results

Effect of temperature upon sensitivity of mouse vas deferens to noradrenaline, methoxamine and UK 14304. Lowering the bath temperature from 37 to 20 or 12°C produced supersensitivity of the isolated mouse vas deferens, as shown by leftward displacement of the concentration-response curves for NA (Fig. 1A) and for methoxamine (Fig. 1B). The EC50 values for NA obtained at 20 and 12°C were significantly different from those obtained at 37°C; the EC50 values were 6·0 and 10·5 times lower at 20 and at 12°C, respectively.

In the presence of cocaine the EC50 values at 20 and at 12°C were significantly lower than EC50 values at 37°C, namely 2·3 and 3·1 times, respectively. After blockade of neuronal uptake, extraneuronal uptake and β-adrenoceptors (C+H+P), hypothermia still causes supersensitivity. The EC50 values at 20 and 12°C were significantly lower than EC50 values at 37°C: 2·0 and 2·8 times respectively (Table 1). The EC50 values obtained for methoxamine at 20 and 12°C were significantly lower than those

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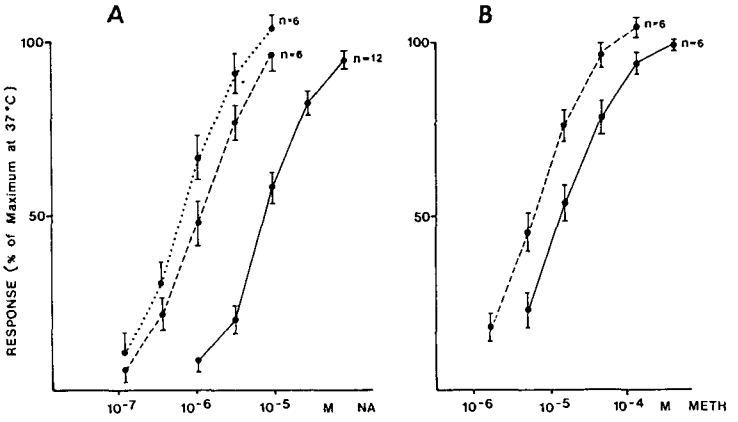

FIG. 1. Concentration-response curves: in A for noradrenaline at 37°C (unbroken line), 20°C (broken line) and 12°C (dotted line); in B for methoxamine at 37°C (unbroken line) and 20°C (broken line).

obtained at 30°C: 2·2 and 2·3 times, respectively (Table 1). In control experiments there were no significant changes in the sensitivity of the tissue throughout the experiment. Hypothermia caused a significant increase of the maximal response. For NA the maximal response at 20 and 12°C were 109±3% and 113±5% of the maximal responses at 37°C, respectively (n=6). For methoxamine the maximal responses at 20 and at 12°C were 107±3% and 109±4% of the maximal response at 37°C, respectively (n=6).

UK 14304 had no effect on mouse vas deferens at 37°C (concentrations from 5 nM up to 20 μM were assayed). At 20°C UK 14304 caused small contractions in most of the preparations and at 12°C all the preparations responded to the α_2-agonist in concentrations ranging from 5 to 30 nM. These responses were dose related but their magnitude never reached more than 5% of the maximal response to noradrenaline. However, a dose-effect relationship was not possible to establish because there was a desensitization of the tissue. This desensitization was such that when one added more than twice the same doses a progressive reduction of the response was observed. These responses to UK 14304 were not abolished by phenoxybenzamine (100 nM) (Fig. 2).

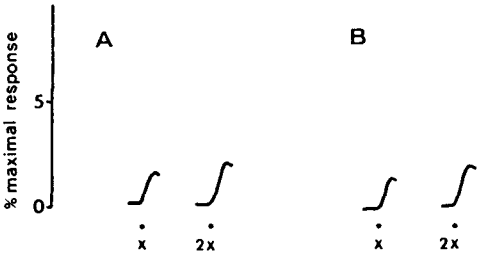

FIG. 2. Typical experiment showing contractile responses to UK 14304 at 12°C of mouse vas deferens: in A, the responses were obtained before and in B after 100 nM phenoxybenzamine; x-10 nM and 2x=20 nM UK 14304.

Effect of temperature on dissociation constants (K_A) of noradrenaline and antagonist dissociation constants (K_B) of prazosin. The dissociation constants (K_A) for the noradrenaline-α-adrenoceptor complex were calculated from the curves obtained before and after treatment with phenoxybenzamine. The mean K_A values of NA at 37°C and at 20°C were 7·5 (4·6–12·2) μM (n=6) and 2·7

Table 1. Effect of temperature on the EC50 values obtained from dose response curves to noradrenaline and methoxamine. EC50 values in μM (geometric means with 95% confidence limits) for noradrenaline (NA) and methoxamine at 37, 20 and 12°C. Values obtained in the absence or in the presence of 12 μM cocaine (C), 60 μM hydrocortisone (H) and 1 μM propranolol (P). R=ratio EC50 at 37°C and EC50 at 20°C; R′=ratio EC50 at 37°C and EC50 at 12°C.

	Temperature °C				
	37	20	R	12	R′
NA	7·56 (6·35–8·58)	1·27* (1·00–1·61)	6·0	0·72* (0·48–0·93)	10·5
NA+C	1·05 (0·78–1·40)	0·46* (0·31–0·68)	2·3	0·34* (0·19–0·61)	3·1
NA+C+H+P	1·27 (0·97–1·66)	0·64* (0·39–1·04)	2·0	0·46* (0·40–0·96)	2·8
Methoxamine	13·40 (8·92–20·10)	6·12* (4·21–8·80)	2·2	5·86* (4·01–8·72)	2·3

* Different from values obtained at 37°C n=6 to 12 experiments. $P<0.05$.

(1·7–3·8) μM (n = 6), respectively. These values differed significantly.

The antagonist dissociation constants (K_B) of prazosin determined at 37 and 20°C were 4·0 (1·8–8·9)nM ($n = 6$) and 1·3 (1·0–1·7) nM (n = 6), respectively. These values differed significantly.

Discussion

Hypothermia-induced potentiation of α-adrenoceptor-mediated responses of the mouse vas deferens to NA is mainly caused by inhibition of sites of loss. However, even after blockade of the sites of loss (neuronal and extraneuronal uptake) in the case of NA, or when using methoxamine, which is not a substrate for uptake or metabolism (Trendelenburg et al 1970) hypothermia still causes a small but significant increase in responsiveness, as we have demonstrated. Moreover, we have shown in earlier experiments (unpublished results) that inhibition of catechol-*O*-methyltransferase and monoaminoxidase, in the presence of cocaine, failed to further alter the potency of NA at 37°C, so that we can exclude the remaining supersensitivity to be due to the inhibition of the metabolizing enzymes by cold. In the rat vas deferens, French et al (1986) showed that there was a slight increase in tissue sensitivity to NA by lowering bath temperature. These authors explained this slight increase as caused by inhibition of the uptake mechanisms, in agreement with the previous report by Buckner et al (1975) from results obtained on the mouse vas deferens. Although we have found that neuronal uptake represents a major pathway which can account for supersensitivity, the remaining supersensitivity induced by cooling can not be attributed to inhibition of sites of loss. This conclusion is supported by the following three facts: supersensitivity to NA after inhibition of all known sites of loss, supersensitivity to methoxamine (which is not a substrate for uptake or metabolism) and changes in sensitivity to UK 14304 (there was no contractile response at 37°C but responses were recorded at 20 or 12°C).

To study the possibility that the remaining supersensitivity was due to an increased receptor affinity, we determined pharmacologically the affinity of NA and prazosin for the α-adrenoceptors. The results obtained confirm this assumption, since they indicate an increase in affinity both for NA and for prazosin.

If we assume that in mouse vas deferens the population of post-junctional α-adrenoceptors belongs to the α_1-subtype (there was no response to UK 14304 at 37°C) our results apparently are difficult to reconcile with those of Vanhoutte & Flavahan (1986). These authors referred that cooling enhances α_2-adrenergic responses (presumably because of an increased receptor affinity) but depress α_1-adrenoceptor responses (presumably because of direct inhibitory effect on the contractile process). However we have found an α_1-adrenoceptor-mediated supersensitivity (methoxamine is a specific α_1-agonist and prazosin is a specific α_1-antagonist); moreover our results are also in favour of an α_2-adrenoceptor-mediated supersensitivity, since tissues exhibited supersensitivity to NA which is a mixed agonist, activating both subtypes of adrenoceptors. Furthermore, a new finding supports this conclusion: the evidence of postjunctional α_2-adrenoceptor population by lowering bath temperature as expressed by the appearance of contractile responses evoked by low concentrations of UK 14304 at 20 and 12°C.

In conclusion, our data suggest that hypothermia-induced supersensitivity to NA is caused in part by inhibition of the removal of the amine from the biophase and in part by an increase in responsiveness which can be attributed to changes in postjunctional α_1- and α_2-adrenoceptors.

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