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Supersensitivity to isoprenaline in right atria isolated from cold-exposed rats

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It is generally accepted that cold exposure increases the cardiovascular effects of catecholamines (Estler & Ammon 1969; Himms-Hagen 1975). However, there are few reports on drug responsiveness of organs and tissues isolated from cold-exposed and/or cold-acclimated animals. Recently, it has been reported that hearts isolated from cold-acclimated rats were not supersensitive to the chronotropic effect of noradrenaline (Östman-Smith 1979). The aim of the present investigation was to examine the responsiveness of right atria, isolated from cold-exposed rats, to the chronotropic effect of isoprenaline. Furthermore, the apparent dissociation constant (K_B) for the competitive antagonist propranolol was determined in an effort to shed light on the role of β-cardiac adrenoceptors in cold-induced increased responsiveness to catecholamines.

Method

Male Wistar rats, 250–300 g were housed individually in a climatized room (22 °C ± 1 °C) with standard laboratory chow and tap water freely available. Lighting operated on a 12 h light/dark cycle with lights on at 12.00 h noon. Rats were allowed 7 days to habituate to the environmental conditions. After this period, the animals were continuously exposed to a temperature of 4 °C, during 1,3, 5 or 7 days. Control rats were kept in the climatized room. Right atria were mounted in 35 ml organ-baths containing Krebs-Henseleit solution of the following composition (mM): NaCl, 115·0; KCl, 4·6; CaCl, 2·5; KH_2PO_4, 1·2; $MgSO_4$, 2·5; $NaHCO_3$, 25·0; glucose 11·0 and ascorbic acid 0·1. The solution was kept at 36·5 °C and continuously gassed with 95% O_2—5% CO_2. The diastolic tension applied to the atria was just enough to permit recording of beats using full transducer sensitivity. A period of 1 h was allowed for equilibration, during which time the organ-baths were drained and refilled with fresh bathing medium at 15 min intervals. This period of time was enough to establish a stable resting rate. Considering that isoprenaline is not a substrate for either MAO or neuronal uptake process (Hertting 1964; Bönisch 1978), but is a substrate for the extraneuronal uptake (Callingham & Burgen 1966) which is well-developed in rat heart (Trendelenburg 1978), all experiments were performed after the tissues were submitted to phenoxybenzamine (10^{-4} M) for 15 min followed by thorough washout. Addition of phenoxybenzamine impairs the extraneuronal uptake and blocks α-adrenoceptors (O'Donnell & Wanstall 1979). However, the antagonist increases the resting rate of the preparation, thereby disturbing the concentration-effect curve to isoprenaline. To prevent this effect of phenoxybenzamine, the tissues, before its addition, were chemically denervated using 6-hydroxydopamine (6-OHDA) according to Aprigliano & Hermsmeyer (1976). To assess the efficiency of 6-OHDA denervation in rat atria noradrenaline content was fluorimetrically determined (Atack & Magnusson 1978). Full cumulative concentration-effect curves to the chronotropic effect of isoprenaline were obtained by stepwise increases in total isoprenaline concentration. Differences in sensitivity were evaluated by comparing pD_2 (i.e. the negative $\log_{10}$ of the molar concentration of isoprenaline producing 50% of the maximum response). Apparent dissociation constant (K_B) of propranolol was determined by the dose-ratio method (Besse & Furchgott 1976). The equation relating K_B of a competitive antagonist to the dose-ratio (DR) and the molar concentration of the antagonist ([B]) is (Besse & Furchgott 1976): $K_B = [B]/(DR-1)$.

DR was determined by comparing geometric mean EC50's values ($pD_2 = -\log EC50$) obtained in the presence and absence of the antagonist. Significance of differences between means were assessed by Student's *t*-test for unpaired samples, analysis of variance (Snedecor 1956) and New Multiple Range Test (Duncan 1955).

Results

In-vitro exposure to 6-OHDA reduces the noradrenaline content of rat atria from 1·02 ± 0·04 μg g^{-1} wet weight of tissue to 0·28 ± 0·03 μg g^{-1} wet weight of tissue ($P < 0·05$). Addition of phenoxybenzamine to 6-OHDA pretreated rat atria did not significantly alter the resting rates of the preparations. Table 1 shows that sensitivity to the chronotropic effects of isoprenaline in isolated right atria significantly increased after 5- or 7-days of cold exposure. The sensitivity to isoprenaline was higher after 5-days than that observed after 7-days of cold exposure. There were no differences ($P > 0·05$) in mean initial rates, and only the maximum rate to isoprenaline in right atria isolated from 7-days cold exposed rats was different ($P < 0·05$) from the maximum rates observed in the control group and after 1, 3 and 5 days of cold exposure. In atria isolated from rats

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Table 1. Chronotropic effect of isoprenaline and K_B values of propranolol in right atria isolated from cold-exposed rats.

Days of cold exposure	Mean initial rate beats min^{-1} (± s.e.)	Mean maximum rate beats min^{-1} (± s.e.)	pD_2[a] (± s.e.)	N[b]	Log ratio[c]	K_B[d] × 10^{-11}M (95% C.I.)	N[b]
—	318 (11)	456 (6)	9.67 (0·20)	5	—	52·2 (37·3–73·1)	5
1	300 (8)	420 (4)	10·05 (0·20)	4	0·38	16·0 (6·0–40·1)	4
3	291 (13)	430 (9)	9.69 (0·12)	4	0·02	136·0 (60·0–290·0)	5
5	305 (5)	452 (2)	12·31 (0·18)*	5	2·64	0·10 (0·04–0·23)*	5
7	308 (13)	412 (12)*	10·76 (0·10)*	5	1·09	2·32 (1·59–5·38)*	5

[a] Negative log of the molar concentration producing 50% of maximum response.
[b] Number of experiments.
[c] pD_2 cold-exposed − pD_2 control.
Braces indicate those parameters which were not significantly different ($P > 0.05$) from each other.
[d] $K_B = \frac{[B]}{DR - 1}$
* Significantly different from control ($P < 0.05$).

exposed to cold for 1 and 3 days the K_B of propranolol did not significantly differ from the K_B value obtained in the control group ($P > 0.05$). However after 5 or 7 days of cold exposure there was a large decrease in K_B values of propranolol ($P < 0.05$).

Discussion

In the present experiment atria isolated from 5 or 7 days cold-exposed rats showed supersensitivity to isoprenaline. This catecholamine is not a substrate for either MAO or neuronal uptake process (Hertting 1964; Bönisch 1978). Possible alterations in other mechanisms for disposition of isoprenaline can be also ruled out from causing supersensitivity since all experiments were performed in atria pretreated with phenoxybenzamine, a potent inhibitor of the extraneuronal uptake process. Therefore, the observed supersensitivity could only result from increased β-adrenoceptor sensitivity and/or post-receptor alterations. Cold exposure for 5 or 7 days also decreased the K_B of propranolol. K_B numerically corresponds to the apparent dissociation constant of a competitive antagonist and its inverse indicates the affinity constant of a receptor population for a competitive antagonist (Besse & Furchgott 1976). Consequently, K_B is not affected by alterations located beyond drug-receptor interaction or changes in the density of the receptor population, not excluding, however, the possibility that post-receptor events are involved in cold-induced supersensitivity to isoprenaline.

In summary, the present report demonstrates that cold exposure for 5 or 7 days elicited supersensitivity to the chronotropic effect of isoprenaline in isolated right atria and it is suggested that, at least partially, an increased affinity of β-adrenoceptors contributes to the supersensitivity. The molecular mechanisms of the cold-induced alteration of the affinity of cardiac β-adrenoceptors for the competitive antagonist are unknown. However, the present results seems to indicate an important functional role for cardiac β-adrenoceptors in the mechanisms of resistance and adaptation to cold.

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