Heterogeneity of Beta Adrenoceptors in Right Atria Isolated from Cold-Exposed Rats

MARIA LUCIA CALLIA and SERGIO DE MORAES
Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brasil
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ABSTRACT
The chronotropic response of right atria isolated from 5-day-cold-exposed rats to isoproterenol and norepinephrine was studied. A large increase in the sensitivity of the pacemaker to isoproterenol and a decrease in the sensitivity to norepinephrine occurred. Determination of pA2 values of propranolol and metoprolol using isoproterenol and norepinephrine as agonists and analysis of the slopes of Schild plots suggested that in atria isolated from control rats the chronotropic effect of isoproterenol and norepinephrine resulted from the preferential interaction of the catecholamines with a homogenous beta-1 adrenoceptor population. After cold exposure the affinity of atrial adrenoceptors for propranolol increased when the agonist was isoproterenol and decreased when norepinephrine was used. The slopes of the Schild plots of metoprolol when the agonists were isoproterenol or norepinephrine were not unitary unless the experiments were performed in the presence of butoxamine. However, butoxamine prevented the demonstration of cold-induced supersensitivity to isoproterenol, leaving the subsensitivity to norepinephrine unaffected. It is concluded that cold-induced heterogeneity of the atrial beta adrenoceptors is responsible for the increased sensitivity to isoproterenol. Probably, subsensitivity to norepinephrine resulted from conformational alterations of the atrial beta-1 adrenoceptors.

It is generally accepted that, at least in in vivo experiments, cold exposure increases the responsiveness to the metabolic and cardiovascular effects of catecholamines (Estler and Ammon, 1969; Fregly et al., 1977; Barney et al., 1980). However, there is a paucity of information concerning the effect of cold exposure on the responsiveness of the isolated rat pacemaker to the chronotropic effect of catecholamines and the few reports are controversial. It has been reported that 7 days of cold exposure decreased the sensitivity of the rat isolated right atrium to the chronotropic effect of norepinephrine and phenylephrine, whereas the sensitivity to isoproterenol remained unaltered (Harri et al., 1974). On the other hand, cold exposure for 5 or 7 days induced supersensitivity of the isolated rat pacemaker to isoproterenol (Callia and de Moraes, 1983). A hypothesis concerning the mechanism by which cold exposure produces changes in the sensitivity to the chronotropic effect of catecholamines is an alteration in the properties of atrial beta adrenoceptors. Preliminary experiments revealed that 5 days of cold exposure increased the affinity of rat atrial beta adrenoceptors for propranolol (Callia and de Moraes, 1983).

In the present experiments an attempt is made to clarify the effects of cold exposure on the sensitivity of the isolated rat pacemaker to isoproterenol and norepinephrine. Our results showed that cold-induced sensitivity changes to the two catecholamines could result from heterogeneity of the atrial beta adrenoceptors and/or conformational alterations of the beta-1 adrenoceptor population.

Methods
General considerations. Male Wistar rats weighing 250 to 300 g were used. The animals were housed, in groups of three, in a climatized room (22°C ± 1°C) with standard laboratory chow and tap water freely available. Lighting operated on a 12 hr light/dark cycle with lights on at 12 noon. After 7 days of habituation to the environmental conditions the animals were continuously exposed to a temperature of 4°C for 5 days. Control rats were kept in the climatized room (22°C ± 1°C). The animals were killed by stunning and bleeding and atria were suspended in 35-ml organ baths containing Krebs-Henseleit solution of the following composition (millimolar): NaCl, 115.0; KCl, 4.6; CaCl₂, 2.5; KH₂PO₄, 1.2; MgSO₄, 2.5; NaHCO₃, 25.0; glucose 11.0; and ascorbic acid, 0.1. The solution was kept at 36.5°C and gassed with 95% O₂-5% CO₂ (pH 7.4). Atria were attached to isometric transducers coupled to a Narco Bio-Systems polygraph recorder. The diastolic tension applied to the atria was just enough to permit a pen deflection of 0.5 cm/beat using full transducer sensitivity (Buckner et al., 1978). A period of 1 hr 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. All experiments were carried out after the tissues had been exposed to phenoxymenzamine (100 μM) for 15 min to block alpha adrenoceptors and the extraneuronal uptake (O'Donnell and Wanstall, 1979). Addi-

ABBREVIATIONS: 6-OHDA, 6-hydroxydopamine; A-S plots, Schild plots.
tion of phenoxybenzamine was followed by 30 min of thorough washout period. Nevertheless, the resting rates of the preparations increased. To prevent this effect of the antagonist, atria were chemically denervated using 6-OHDA according to Aprigliano and Hermansyver (1976). To assess the efficiency of 6-OHDA (300 μg/ml) pretreatment, endogenous norepinephrine content was determined fluorimetrically (Attack and Magnusson, 1978). When norepinephrine was used atria were exposed continuously to cocaine (10 μM).

Full cumulative concentration-effect curves to the chronotropic effect of isoproterenol or norepinephrine were obtained by stepwise increases (0.5 log unit) in the agonist concentrations. Only one concentration-effect curve was obtained with each pair of atria. The agonist concentration producing a response which was 50% of maximum (EC50) in individual experiments was calculated and presented as mean negative logarithms (pD2).

Determination of pA2 values of propranolol and metoprolol. pA2 values of propranolol, a nonselective β-adrenoceptor antagonist, and for metoprolol, a selective β1-adrenoceptor antagonist, were determined according to Arunlakshana and Schild (1959). A full concentration-effect curve to isoproterenol or norepinephrine was obtained, as described above. After thorough washout, atria were incubated with propranolol or metoprolol (1 nM–1 μM) for 60 min. In the presence of the antagonist, a new concentration-effect curve to norepinephrine or isoproterenol was obtained and the dose ratio (EC50 of the agonist in the presence of the antagonist divided by the EC50 in the absence of the antagonist) was determined. According to Arunlakshana and Schild (1959), when the blockade is competitive a plot relating the logarithms of (dose ratio–1) against the negative logarithms of the antagonist molar concentrations should generate a straight line with an unitary slope and intercept along the abcissa of pA2. To analyze the possible influence of a β2-adrenoceptor population on the determination of the pA2 of metoprolol, using as the agonist isoproterenol, experiments also were performed after the exposure of the tissues to the selective β2-adrenoceptor antagonist butoxamine (3 μM) for 60 min.

Determination of plasma corticosterone levels. Plasma corticosterone was determined fluorimetrically (Mattingly, 1962) in control and cold-exposed rats. The animals were anesthetized with sodium pentobarbital (40 mg/kg i.p.) and ether. After 30 min of anesthesia, blood samples (3–4 ml) were collected from the renal vein and centrifuged for 15 min (10,000 r.p.m.) at 5°C. Plasma (0.5 ml) was transferred to 5 ml of dichloromethane and steroids other than corticosterone were destroyed by the addition of 0.5 ml of 0.1 N NaOH. Corticosterone was converted to a fluorescent product by the addition of 3 ml of sulfuric acid-ethanol (7:3). Fluorescence was measured in a Perkin-Elmer fluorescence spectrophotometer model 204-A, with excitation at 470 nm and emission at 520 nm. Blanks and internal standards were used and recovery was 98%.

Statistical analysis. Statistical differences between two means (P < .05) were determined by Student's t test for unpaired samples or by testing for overlap of 95% confidence intervals (Snedecor and Cochran, 1967). A-S plots were drawn by linear regression calculated by the method of least-squares.

Drugs. All drug solutions were prepared daily in deionized water and stored in ice. Phenoxybenzamine was dissolved in acidified ethanol and diluted to the appropriate concentration in Krebs-Henseleit solution. (-)-Norepinephrine hydrochloride, (+)-isoproterenol sulfate, (+)-propranolol hydrochloride, 6-OHDA hydrobromide and metoprolol hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO). Cocaine hydrochloride and phenoxybenzamine were purchased from Merck A-G (Darmstadt, FRG) and Smith Kline and French Laboratories (Philadelphia, PA), respectively.

Results

Plasma corticosterone levels increased only after 1 day of cold exposure (control, 11.6 μg/100 ml of plasma with 95% confidence intervals of 8.7 and 14.5; cold-exposed, 32.0 μg/100 ml of plasma with 95% confidence intervals of 21.8 and 42.2, P < .05) at a time when there was only a slight increase in the sensitivity of the pacemaker to isoproterenol (Callia and de Moraes, 1983). In the subsequent days of cold exposure there was a return of plasma corticosterone levels toward the control value with the lowest plasma concentration of corticosterone being observed after 5 days of cold exposure (9.9 μg/100 ml of plasma with 95% confidence intervals of 4.3 and 15.6, P > .05 in relation to the control group). In vitro exposure to 6-OHDA reduced the norepinephrine content of rat atria from 1.02 ± 0.04 μg/g of wet weight of tissue to 0.28 ± 0.03 μg/g of wet weight of tissue (P < .05). The subsequent addition of phenoxybenzamine and cocaine to previously in vitro denervated atria did not alter significantly the resting rate of the preparations (P > .05).

The effect of cold exposure on the chronotropic response of the rat pacemaker to isoproterenol is illustrated in figure 1. Cold exposure resulted in a 2.89 log shift (766-fold at the EC50 level) of the concentration-effect curve to isoproterenol without any significant alteration of the resting rate (control, 318 ± 11 beats/min; cold-exposed, 305 ± 5 beats/min, P > .05) or maximum response (control, 456 ± 6 beats/min; cold-exposed, 412 ± 12 beats/min, P > .05). Addition of butoxamine (10-6 M), a selective β2-adrenoceptor antagonist, prevented the demonstration of supersensitivity to isoproterenol in atria isolated from cold-exposed rats. However, 5 days of cold exposure induced subsensitivity to norepinephrine (3.5-fold at the EC50 level, P < .05) with no significant changes of atrial resting rate (control, 322 ± 7 beats/min; cold-exposed, 434 ± 10 beats/min, P > .05). Cold-induced subsensitivity to norepinephrine was not affected by the previous addition of 3 μM butoxamine (fig. 2). These results are summarized in table 1.

Figure 3 and table 2 show pA2 values and slopes of A-S plots of propranolol using isoproterenol and norepinephrine as agonists. Atria isolated from the control group showed pA2 values of propranolol which were independent of the β2-adrenoceptor selectivity of the agonist and slopes of A-S plots which did not differ from 1.0 (P > .05). However, atria isolated from 5-day cold-exposed rats showed a statistically significant increase (562-fold) in the pA2 value of propranolol when isoproterenol was used as the agonist and a decrease in the pA2 value of

![Fig. 1. Mean concentration-effect curves for the chronotropic effect of isoproterenol obtained in right atria isolated from control and 5-day cold-exposed rats. Some experiments using atria isolated from cold-exposed rats were performed in the presence of butoxamine (3 μM). Vertical bars represent S.E.S.](image-url)
propranolol when norepinephrine was used (5.8-fold), although with both agonists the slopes of A-S plots did not differ from 1.0 (P > .05).

pA₂ values and slopes of A-S plots of metoprolol vs. isoproterenol and norepinephrine were obtained in atria isolated from the control and cold-exposed groups (fig. 4, table 3). Again, pA₂ values and slopes of A-S plots (which were not different from 1.0, P > .05) did not depend on the beta adrenoceptor selectivity of the agonist in atria isolated from the control group. However, in atria isolated from 5-day-cold-exposed rats pA₂ values of metoprolol remained undetermined because the slopes of A-S plots were significantly different from 1.0 (P < .05). When butoxamine (3 μM) was added to the bathing medium, 60 min before the addition of metoprolol, a slope which did not differ from 1.0 (P > .05) was produced. The pA₂ value of metoprolol, obtained in atria isolated from cold-exposed rats, increased in the presence of 3 μM butoxamine (6.4-fold) indicating a small increased affinity of the beta-1 adrenoceptor population for metoprolol.

**Discussion**

We have shown recently that atria isolated from 5- or 7-day-cold-exposed rats showed supersensitivity to the chronotropic effect of isoproterenol (Callia and de Moraes, 1983). The present findings demonstrate the simultaneous presence of sensivity to norepinephrine in atria isolated from rats exposed to cold for 5 days. Possible alterations in the mechanisms of disposition of isoproterenol or norepinephrine can be ruled out as causing sensitivity changes to the catecholamines because all experiments were carried out in chemically denervated atria and in the presence of phenoxbenzamine and cocaine. Therefore, the observed changes in sensitivity could possibly result from alterations in the atrial beta adrenoceptors and/or changes located beyond the receptor level. Beta adrenoceptors have been classified into two subtypes based on differences in the selective of the receptor-mediated responses. Beta-1 adrenoceptors have a higher sensitivity for isoproterenol than for norepinephrine and epinephrine whereas beta-2 adrenoceptors have a higher sensitivity for epinephrine than for norepinephrine (Lands et al., 1967a,b). Pharmacological in vitro studies in rat heart have shown that the chronotropic effects of isoproterenol and norepinephrine are mediated by a homogeneous population of beta-1 adrenoceptors (Bryan et al., 1981). However, recent radioligand binding analyses have demonstrated the presence of a comparatively small population of beta-2 adrenoceptor subtypes, that seem to mediate the same physiological response as that mediated by the beta-1 adrenoceptor subtypes (Minneman et al., 1979; Minneman and Molinoff, 1980). In the present experiments, it has been clearly confirmed that in atria isolated from control rats the chronotropic effect of isoproterenol and norepinephrine was mediated by a homogeneous population of beta-1 adrenoceptors. The pA₂ value of metoprolol, a selective beta-1 adrenoceptor antagonist, was not influenced by the selectivity of the agonist and the slope of A-S plot was consistently 1.0 when isoproterenol or norepinephrine were used. This is undisputed evidence of the pharmacological homogeneity of receptor populations (Furchgott, 1976; Taylor, 1982; Kenakin, 1982). It has been reported previously that cold exposure for 5 days decreased the pA₂ value of propranolol (Callia and de Moraes, 1983). pA₂ corresponds numerically to the apparent dissociation constant of a competitive antagonist and its inverse indicates the affinity constant of the receptor.
TABLE 2

<table>
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<th>Agonist</th>
<th>Control</th>
<th>Cold-Exposed</th>
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<tbody>
<tr>
<td></td>
<td>$p_A_2$ (±S.E.M.)</td>
<td>Slope$^a$</td>
</tr>
<tr>
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<td>9.20 ± 0.04</td>
<td>0.95 (0.83-1.06)</td>
</tr>
<tr>
<td>NE</td>
<td>9.19 ± 0.03</td>
<td>0.95 (0.90-1.0)</td>
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</table>

* Mean value calculated from individual $p_A_2$ obtained from data in figure 3 using the equation, $p_A_2 = \log (DR - 1) - \log [B]$ (MacKay, 1978).
* Mean value and 95% confidence intervals.
* Number of experiments.
* Significantly different from the control group ($P < .05$).

Fig. 4. A-S plots for the antagonism of norepinephrine (■, △) and isoproterenol (■, ▽, △) by metoprolol in right atria isolated from control and cold-exposed rats. Some experiments using atria isolated from cold-exposed rats were performed in the presence of butoxamine (3 μM), using isoproterenol as the agonist. The lines represent the best fit obtained by linear least-squares regression analysis for the combined data from number of points and animals shown in table 3.

population for the antagonist (Besse and Furchgott, 1976). It is accepted generally that when a heterogeneous receptor population is activated by a nonselective agonist in a certain tissue, then the A-S plot for an antagonist, which is selective for one of the receptor subtypes, will have a slope of less than 1.0 (Furchgott, 1978; Harper and Hughes, 1978; O'Donnell and Wanstall, 1979; Kenakin, 1982). In atria isolated from rats exposed to cold for 5 days, the slopes of the A-S plots drawn for metoprolol were consistently less than 1.0 when isoproterenol or norepinephrine were used as the agonist, suggesting the presence of a heterogeneous beta adrenoceptor population. Moreover, $p_A_2$ of propranolol decreased when the agonist was isoproterenol and increased when the agonist was norepinephrine. $p_A_2$ of metoprolol increased when the agonist was isoproterenol in atria isolated from cold-exposed rats and pre-exposed to butoxamine. Thus, in atria isolated from rats exposed to cold for 5 days, $p_A_2$ values of a nonselective antagonist, as propranolol, was dependent on the selectivity of the agonist used strongly suggesting, as previously proposed (O'Donnell and Wanstall, 1979), the presence of a heterogeneous population of beta adrenoceptors in atria isolated from rats exposed to cold for 5 days.

An intriguing hypothesis has to be put forward now. Is this cold-induced mixed population of beta adrenoceptors causally related to the supersensitivity to isoproterenol? The use of butoxamine, a selective beta-2 adrenoceptor blocking drug, seems to confirm this hypothesis. Addition of butoxamine suppressed the cold-induced supersensitivity to isoproterenol leaving unaltered the decreased responsiveness to norepinephrine. This result strongly suggests that cold exposure activates the beta-2 adrenoceptor population that was pharmacologically irrelevant in atria isolated from control rats.

Norepinephrine is a beta-1 selective agonist that has a small affinity and efficacy at beta-2 adrenoceptors. Consequently, cold-induced beta-2 adrenoceptors also should result in an increased responsiveness to the neurotransmitter. However, there was subsensitivity to norepinephrine after cold exposure. An important heuristic aspect of the use of $p_A_2$ values for the identification and classification of receptor subtypes is that identical receptors should give rise to the same $p_A_2$ value for a given pair of agonists and antagonists. After cold exposure the affinity of the atrial adrenoceptor population for propranolol decreased when the agonist was norepinephrine (see table 2). Conversely, the affinity of the adrenoceptor population for

TABLE 3

<table>
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<th>Agonist</th>
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<th>Cold-Exposed</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>$p_A_2$ (±S.E.M.)</td>
<td>Slope$^a$</td>
</tr>
<tr>
<td>ISO</td>
<td>8.37 (0.03)</td>
<td>1.05 (0.69-1.33)</td>
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<tr>
<td>NE</td>
<td>8.27 (0.02)</td>
<td>1.02 (0.82-1.21)</td>
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<tr>
<td></td>
<td>ISO</td>
<td>9.18 (0.03)$^*$</td>
</tr>
</tbody>
</table>

* Mean value calculated from individual $p_A_2$ values obtained from data in figure 4 using the equation, $p_A_2 = \log (DR - 1) - \log [B]$ (MacKay, 1978).
* Mean value and 95% confidence intervals.
* Number of experiments.
* Significantly different from 1.0 ($P < .05$). * $p_A_2$ values and slope were obtained in the presence of butoxamine (3 μM). Significantly different from the control group in the absence of butoxamine ($P < .05$).
metropolrol increased when the experiments were carried out in the presence of butoxamine (see table 3). Taken together, these data, although not extensive enough, seem to indicate that conformational alterations of the atrial beta-1 adrenoceptors could be responsible for the subsensitivity to norepinephrine. This point of view is strengthened by the lack of effect of butoxamine in suppressing the cold-induced desensitization to the neurotransmitter, apparently ruling out any role of the beta-2 adrenoceptor population in the subsensitivity to norepinephrine observed after 5 days of cold exposure.

Recently, it was shown that prolonged glucocorticoid administration induced an enhanced density of beta adrenoceptors in rat lung membranes (Mano et al., 1979; Scarpace and Abrass, 1982). Methylprednisolone pretreatment was associated with an increased beta adrenoceptor density in rat lung membranes in which beta adrenoceptors have been down-regulated by pretreatment with metaproterenol, a beta adrenoceptor agonist (Scarpace and Abrass, 1982). It is tempting to look for a causal relationship between cold-induced heterogeneity of the atrial beta adrenoceptors and the enhanced plasma corticosterone level observed during cold exposure. However, the plasma level of the steroid increased only after 1 day of cold exposure. Furthermore, supersensitivity to isoproterenol appeared only after 5 days of cold exposure, at a time when there is no difference in plasma levels of corticosterone between control and cold-exposed rats.

The exact mechanisms involved in this alteration of the atrial beta adrenoceptor properties observed after 5 days of cold exposure remain obscure. Nevertheless, the present results seem to indicate an important functional role for the cardiac beta adrenoceptors in the homeostatic mechanisms controlling cell sensitivity during the initial process of resistance and adaptation to cold.

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References


Send reprint requests to: Dr. S. de Moraes, Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo Cidade Universitária, Curitiba, Cep. 80505, São Paulo, Brazil.