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The effects of acute reserpine administration on the sensitivity of the isolated pacemaker from rat heart to isoprenaline and noradrenaline

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The effects of reserpine on the sensitivity of the isolated pacemaker from rat heart to the chronotropic effect of isoprenaline and noradrenaline were studied. A single large dose of reserpine (2·5 mg kg^{-1}) administered to rats 24 h before killing induces supersensitivity of the isolated pacemaker to isoprenaline, leaving unaltered the responsiveness of the pacemaker to noradrenaline. Reserpine at the dose of 1·0 mg kg^{-1} did not alter the sensitivity of the pacemaker to the catecholamines. Only the larger dose of reserpine raised the corticosterone plasma level. It is possible that a corticosterone-mediated inhibition of the extraneuronal uptake process is responsible for the supersensitivity to isoprenaline. Large doses of reserpine should not be used in experiments aimed to study cardiac sensitivity to isoprenaline or extraneuronal uptake and metabolism of the catecholamine.

Although it is generally accepted that a single dose of reserpine has no effect on the responsiveness of isolated tissues to catecholamines (Trendelenburg 1966; Fleming et al 1973; Fleming 1976) there are reports to the contrary. It has been reported that a single large dose of reserpine (5 mg kg^{-1}) causes supersensitivity of the rat isolated uterus to the inhibitory effect of isoprenaline (Tozzi 1973). Gibson & Pollock (1975) noticed that an acute low dose of reserpine (1 mg kg^{-1}) increased the maximum response of the rat isolated anoccocygeus muscle to noradrenaline. It has been reported that the pretreatment of guinea-pigs with one very low dose of reserpine (0·1 mg kg^{-1}) produced a slight, but significant, increase in the sensitivity of the isolated pacemaker to the chronotropic effect of isoprenaline (Torphy et al 1982). In the present communication, an attempt was made to address the controversy outlined above. The approach was to evaluate the chronotropic effect of isoprenaline and noradrenaline in right atria isolated from rats acutely pretreated with reserpine.

Materials and methods

Male Wistar rats, 200 to 250 g, were pretreated with reserpine (1·0 mg kg^{-1} or 2·5 mg kg^{-1} i.p.) dissolved in a 20% solution of ascorbic acid 24 h before death. Control rats received only the vehicle. The animals were killed by a sharp blow on the head and bleeding. Right atria were immediately excised and set up for isometric recording of spontaneous beating in physiological salt solution of the following mM composition: NaCl, 115·0; KCl, 4·6; $CaCl_2$, 2·5; KH_2PO_4, 1·2; $MgSO_4.7H_2O$, 2·5; $NaHCO_3$, 25·0 and glucose, 11·0. To reduce oxidation of the catecholamines, 0·11 mM of ascorbic acid was added. Diastolic tension was just enough to permit a polygraph pen deflection of 0·5 cm beat^{-1} using full transducer sensitivity (Bückner et al 1978). The bathing medium was kept at 36·5 °C and continuously gassed with 95% O_2–5% CO_2. Atria were set up in 30 mL organ baths with changes of the bathing medium at 15 min intervals until their spontaneous rate did not change by more than 5 beats min^{-1}. This took about 45 min. Full cumulative concentration–effect curves to isoprenaline or noradrenaline were obtained by stepwise increases (0·5 log units) in the agonist concentration. Only one concentration–effect curve was obtained with each atrium. The agonist concentration producing a response which was 50% of maximum (EC50) was calculated and mean EC50 values are presented as geometric means with 95% confidence intervals (Fleming et al 1972). Plasma corticosterone levels were determined according to the method of Mattingly (1962) as described elsewhere (Callia & De Moraes 1984). Results were analysed by Student's *t*-test for unpaired samples (Snedecor & Cochran 1967).

Results and discussion

Table 1 shows the effect of acute reserpine administration on the sensitivity and maximum response of the isolated pacemaker of rat heart to the chronotropic effect of isoprenaline and noradrenaline. Right atria isolated from rats that were pretreated with 2·5 mg kg^{-1} of reserpine showed supersensitivity to isoprenaline as evidenced by a 18·0-fold decrease in the EC50 value ($P < 0·01$) without any significant alteration of the pacemaker maximum response to this catecholamine ($P > 0·05$). However, 2·5 mg kg^{-1} of reserpine did not change any of the parameters of the concentration–effect curve to noradrenaline. A smaller dose of reserpine (1·0 mg kg^{-1}) did not significantly alter sensitivity of the pacemaker to isoprenaline (control: 1·8 nM with confidence intervals of 1·7 and 1·9 and reserpine-pretreated: 1·4 nM with confidence intervals of 0·9 and 2·3; $P > 0·05$). Only the larger dose of reserpine induced a 63·4% increase in the plasma corticosterone level (control: 10·25 ± 1·89 μg/100 mL of plasma; reserpine, 2·5 mg kg^{-1}: 16·75 ± 2·04 μg/100 mL of plasma and reserpine, 1·0 mg kg^{-1}; 9·09 ± 2·66 μg/100 mL of plasma).

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Table 1. Parameters of the concentration–effect curves to isoprenaline and noradrenaline of rat isolated pacemaker after administration of 2·5 mg kg^{-1} of reserpine.

Agent	Treatment	EC50[a] (95% C.I.)	EC50 Vehicle / EC50 Reserpine	I.R.[b] Beats min^{-1} (s.e.m.)	M.R.[c] Beats min^{-1} (s.e.m.)	N[d]
Isoprenaline	Vehicle	1·8 (1·7–1·9) nM	—	290 (12)	440 (12)	6
	Reserpine	0·1 (0·04–0·5) nM*	18·0	316 (8)	443 (13)	6
Noradrenaline	Vehicle	39·2 (38·4–41) nM	—	318 (9)	450 (2)	6
	Reserpine	23·3 (16·0–34·6) nM	1·6	290 (14)	418 (8)	6

[a] Geometric mean with 95% confidence interval.
[b] Initial rate.
[c] Maximum response.
[d] Number of experiments.
* Significantly different from the vehicle-treated group ($P < 0·01$).

The present results show that an acute large dose of reserpine (2·5 mg kg^{-1}) enhances the sensitivity of the isolated pacemaker to the chronotropic effect of isoprenaline, leaving unaltered the pacemaker responsiveness to noradrenaline. This result seems to rule out any reserpine-induced alteration located at the pacemaker postjunctional β-adrenoceptor population or at the adenylate cyclase subunits as previously suggested (Chiu 1978). Possible modifications of prejunctional dissipating mechanisms should be excluded since there is no reserpine-induced supersensitivity to noradrenaline. Moreover, isoprenaline is not a substrate of the neuronal uptake process (Callingham & Burgen 1966). As previously reported, a single large dose of reserpine induces a persistent increase in the plasma corticosterone level (Brody et al 1961). Corticosterone is a highly efficient in-vitro inhibitor of the extraneuronal uptake (Iversen & Salt 1970) and it is well-known that an in-vitro impairment of the extraneuronal uptake causes supersensitivity to isoprenaline. Apparently, there is a direct relationship between reserpine-induced increase in the plasma corticosterone level and supersensitivity (Gibson & Pollock 1975). An acute dose of reserpine (1·0 mg kg^{-1}) which did not enhance the steroid plasma level did not induce supersensitivity of the pacemaker to isoprenaline. An interesting observation has been recently reported by Morton (1985) who suggested that a corticosterone-sensitive component of the rat atrium extraneuronal uptake is suppressed by prolonged reserpine treatment. Furthermore, an acute injection of reserpine (1 mg kg^{-1}, 1 day) had no effect on the extraneuronal accumulation of [^{3}H]isoprenaline into rat atria. In the present report, the absence of supersensitivity to isoprenaline after administration of 1 mg kg^{-1} of reserpine provides an indirect pharmacological support for Morton's observation. However, a larger dose of reserpine (2·5 mg kg^{-1}, 1 day) did increase the sensitivity of the pacemaker to isoprenaline. Since we have shown that this dose of reserpine induces a persistent increase in plasma corticosterone level, it is conceivable that a reserpine-induced in the plasma corticosterone level may result in a partial inhibition of the extraneuronal uptake process and consequently in supersensitivity to isoprenaline. As demonstrated by Morton (1985) the time-dependent reserpine-induced decrease in the accumulation of [^{3}H]isoprenaline was not due to changes in water balance, ion distribution, extracellular space, effux of [^{3}H]isoprenaline from the atrial tissue, tissue atrophy or a direct effect of reserpine on the extraneuronal uptake process. We show that, at least in pharmacological experiments, the effect of reserpine on the sensitivity of the rat isolated pacemaker to isoprenaline is not time-dependent but is dose-dependent and that apparently there is a direct relationship between reserpine-induced supersensitivity to isoprenaline and reserpine-induced increase in the plasma corticosterone level. However, further experiments are needed to clarify the mechanisms of pacemaker supersensitivity to isoprenaline after a single large dose of reserpine. Nevertheless, the authors recommend that single large doses of reserpine should not be used in experiments aimed to evaluate sensitivity to isoprenaline and/or extraneuronal uptake activity and metabolism of isoprenaline in rat heart.

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Stereoselective blockade of central [^{3}H]5-hydroxytryptamine binding to multiple sites (5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C}) by mianserin and propranolol

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The interaction of the enantiomers of mianserin and propranolol with the binding of [^{3}H]5-hydroxytryptamine ([^{3}H]5-HT) to the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} sites, and with the binding of [^{3}H]ketanserin to the 5-HT_2 site, has been evaluated in rat brain membranes. A stereoselective interaction at the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} sites was demonstrated for both compounds, with (+)-mianserin being a more potent displacer than (−)-mianserin and (−)-propranolol being more potent than (+)-propranolol. Only mianserin interacted in a stereoselective manner with the 5-HT_2 site, (+)-mianserin being the more potent isomer. The stereoselective association of mianserin and propranolol with the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} sites may prove useful in the characterization of these sites.

The heterogeneity of brain 5-hydroxytryptamine (5-HT) receptors has been supported by many studies. Peroutka & Snyder (1979) classified 5-HT receptor sites into 5-HT_1 (labelled by [^{3}H]5-HT) and 5-HT_2 (labelled by [^{3}H]spiperone). More recently, by means of radioligand binding techniques, distinct subtypes of the 5-HT_1 receptor have been demonstrated and termed the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} sites (Hoyer et al 1985). These sites have been shown to satisfy some of the criteria for neurotransmitter receptor binding sites: they are saturable, possess a high affinity for the neurotransmitter 5-HT, have differential pharmacological specificities, and are unevenly distributed throughout the brain (Hoyer et al 1985; Alexander et al 1986; Blurton & Wood 1986). The 5-HT_{1A} site has high affinity for 8-hydroxy-2-(di-*N*-propylamino)tetralin (8-OH-DPAT) and spiperone; the 5-HT_{1B} site displays high affinity for methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole (RU 24969) and propranolol; and the 5-HT_{1C} site shows high affinity for mianserin and mesulergine. The hippocampus is rich in 5-HT_{1A} sites, whereas the striatum is rich in 5-HT_{1B} and 5-HT_{1C} sites. Another important consideration in the identification of neurotransmitter receptor binding sites is that of stereospecificity. Both propranolol and mianserin interact with differing potencies at the three subsites, and both possess an optically active chiral centre. We have therefore examined the interaction of the enantiomers of mianserin and propranolol with the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} subsites, and also with the 5-HT_2 site, in rat brain membranes.

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Materials and methods

[^{3}H]5-HT binding. The binding of [^{3}H]5-HT was as described by Blurton & Wood (1986). The hippocampus and striatum were dissected from 12 male Sprague-Dawley rats (200–250 g) and homogenized in 50 vol Tris-HCl buffer (50 mM, pH 7·4 at 37 °C) using a Polytron (setting 5 for 30 s). The homogenates were centrifuged (40 000*g* for 10 min) and the pellets washed twice by centrifugation and resuspension. After the second wash, the pellet was resuspended in 100 vol Tris-HCl buffer and incubated at 37 °C for 15 min before centrifugation to remove endogenous 5-HT. The final pellet was resuspended in 35 vol Tris-HCl buffer containing 10 μM pargyline, 4 mM $CaCl_2$ and 0·1% ascorbic acid. Throughout the procedure the tissue was kept at 0–4 °C unless otherwise stated.

Tissue homogenate (350 μL), [^{3}H]5-HT (2 nM final concentration, 50 μL), buffer or specific binding displacer (50 μL) and the appropriate concentrations of drug (50 μL) were incubated at 37 °C for 12 min. Incubations were terminated by rapid filtration through Whatman GF/B filters under reduced pressure using a 24 place cell harvester (Brandel, USA). The filters were washed twice with 7·5 mL ice-cold Tris-HCl buffer. Retained radioactivity was measured after extraction into NE266 (Nuclear Enterprise) liquid scintillator at an efficiency of 40–45%. Binding to the 1A site was studied in hippocampal membranes. Drug potencies at the 1B and 1C sites were derived by computer analysis from displacement data in striatal tissue, which were resolved into 1B and 1C components based on the relative proportion of 1B : 1C sites (60 : 40) in this tissue (Blurton & Wood 1986). Specific binding was defined as that displaced by 10^{-5} M 5-HT.

[^{3}H]Ketanserin binding. The binding of [^{3}H]ketanserin was studied in membranes from the rat frontal cortex