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# Catecholamine hypersensitivity of adenylate cyclase after chemical denervation in rat heart

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Trendelenburg [1] in his classical review on supersensitivity to sympathetic amines discussed several mechanisms which could lead to hypersensitivity, among them relationship between supersensitivity and norepinephrine content, deformation of the receptor and release of norepinephrine from nerve endings. Further investigations on these mechanisms carried out in different tissues and animals confirmed the importance of these factors [2–4]. In these investigations the method of chemical denervation with reserpine and 6-hydroxydopamine was widely used during acute and chronic treatments.

The hormonal sensitivity of adenylate cyclase and its close connection with beta-receptor made a good tool of this membrane bound enzyme to investigate receptor sensitivity [5]. Several studies were made concerning the supersensitivity of the adenylate cyclase toward sympathetic amines not only in brain and heart slices [6–8] of rabbit, rat and guinea pig, but also in heart cell membrane preparations from molluscs [9].

The aim of the present work was to compare the effects of reserpine and 6-hydroxydopamine treatment on the catecholamine sensitivity of the adenylate cyclase rat heart particulate cell fractions.

*Materials and methods.* All fine chemicals were from Sigma (St. Louis), labelled compounds were from New England Corp. Boston.

Wistar rats were used for the experiments. 2.5 mg/kg reserpine i.p., were injected 24 hr or 100 mg/kg 6-hydroxydopamine i.p. 2 hr before excising the heart quickly under ether anesthesia. Controls were treated with saline. Ventricles were homogenized in 9 vol. 0.05 M Tris–HCl pH 7.4 buffer containing 0.25 M sucrose using a Potter apparatus at 4°. The homogenate was sedimented at 10000 *g* at 4° for 20 min, washed twice and sedimented again at 10000 *g* with the same volumes of buffer-sucrose. Finally the pellet was suspended in 9 vol. of buffer omitting sucrose. Protein was determined according to Lowry *et al.* [10].

*Adenylate cyclase assay.* The reaction mixture contained 50 mM Tris pH 7.4 buffer, 4 mM theophylline, 2 mM ATP/1 μCi [$^3$H]ATP sp. act. 26 Ci (mM), 2 mM cAMP, 2 mM $MgCl_2$, 6 mg/ml albumine, 1 mM phosphoenolpyruvate and 0.1 mg pyruvate kinase and 100 μl enzyme solution containing 200 μg protein. Incubation was carried out in a final vol. of 300 μl at 37° for 5 and 10 min, c-AMP production was linear during the incubation time. The reaction was stopped by boiling the samples at 100° for 3 min in the presence of a 100 μl recovery mixture (10 mM ATP and 1 mM c-AMP) or by the addition of 200 μl of equal parts of 5% $ZnSO_4$ and 5N $Ba(OH)_2$, 50 μl of the supernatants was applied to Whatman No. 3 MM for paper chromatography as described previously [11]. Radioactivity was measured in a Nuclear-Chicago scintillation spectrometer.

*Results and discussion.* Figure 1 demonstrates adenylate cyclase activity in control and reserpine treated rat ventricles in the presence and absence of $10^{-6}$ and $10^{-5}$ M noradrenaline. The noradrenaline sensitivity with respect to basal activity is increased in the reserpine treated rats, basal activity is significantly lower with respect to the control. Figure 2 shows the changes in adenylate cyclase activity in response to isoproterenol $10^{-5}$ M after 6-hydroxydopamine treatment. There is a significant increase in hormonal stimulation after 6-hydroxydopamine treatment and a decrease in basal activity as well. The decreased basal activity of adenylate cyclase after chemical depletion of catecholamines is observed only in particulate cell fractions whereas in slices no changes in basal activity were observed [3, 12]. Catecholamine concentrations were chosen to give maximal stimulation. No significant differences in noradrenaline $K_a$ values were reported after chemical denervation in brain slices by Vetulani *et al.* [16].

These results seem to emphasize the role of the receptor in short-term supersensitivity since it develops relatively early after denervation, even before the catecholamine content of the heart entirely disappeared. After a single injection of 100 mg/kg 6-OHDA the catecholamine content of the rat heart fell from 0.902 μg/g to 0.042 μg/g w/w within 2 hr [4, 9, 15]. Adenylate cyclase shows supersensitivity not only after *in vitro* noradrenaline stimulation but also after

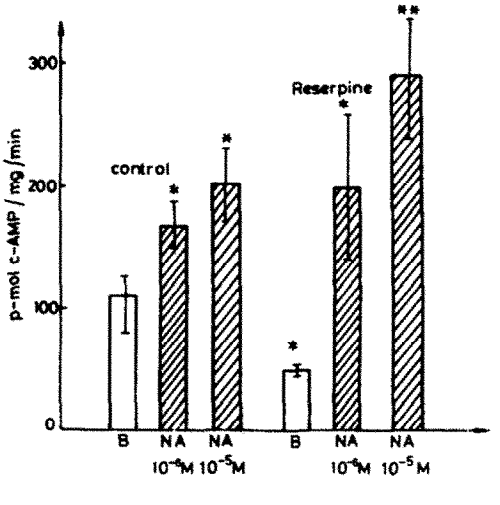


Fig. 1. Adenylate cyclase activity from rat heart ventricle particulate preparations before and after reserpine treatment. B = basal activity, NA = noradrenaline, $n = 5$.

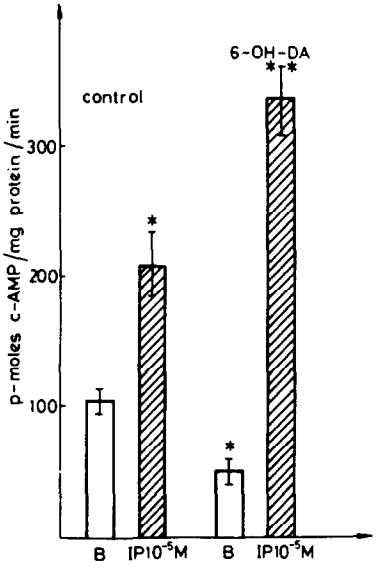


Fig. 2. Adenylate cyclase activity from rat heart ventricle preparations before and after 6-hydroxydopamine treatment. B = basal activity, IP = isoproterenol, $n = 6$. * = $P < 0.01$; ** = $P < 0.001$.

isoproterenol addition to the treated hearts (Fig. 2), which means that it is a typical beta-receptor effect [5]. Similar results were obtained on brain slices and homogenates [6, 7, 12]. We suppose that the lower basal activity after functional denervation was due partly to endogenous catecholamines.

In conclusion one can assume that the beta-receptor hypersensitivity as measured by the activating effect of catecholamines on the adenylate cyclase is a fairly good model of receptor supersensitivity. Short-term desensitization to catecholamines after repeated catecholamine treatment is also reflected in the reduced response of adenylate cyclase activation [13] and therefore the beta-receptor-adenylate-cyclase system is able to feel deviations of catecholamine concentrations in both directions.

This is in contrast with previous observations where supersensitivity was investigated between 4 and 8 days after drug treatment [2, 3, 7, 14] and points to the fact that supersensitivity of adenylate cyclase to catecholamines develops parallel with the decrease of catecholamine concentration, perhaps as quickly as desensitization [13].

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