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THE ADENYLATE CYCLASE ACTIVITY IN HEART MEMBRANES FROM NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS, AFTER CHEMICAL SYMPATHECTOMY, SUGGESTS THE PRESENCE OF PRESYNAPTIC SECRETIN RECEPTORS

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P. CHATELAIN, P. ROBBERECHT, J.C. CAMUS, P. DE NEEF, M. WAELBROECK, J. ROBA and J. CHRISTOPHE, *The adenylate cyclase activity in heart membranes from normotensive and spontaneously hypertensive rats, after chemical sympathectomy, suggests the presence of presynaptic secretin receptors,* European J. Pharmacol. 93 (1983) 271–276.

Normotensive (WKY) and spontaneously hypertensive (SHR) male adult rats were sacrificed 2 and 3 weeks after 6-hydroxydopamine treatment. Untreated WKY and SHR rats served as controls. In rat heart membranes from WKY rats, 6-hydroxydopamine treatment increased guanosine 5′-O-(2-3-imido)-triphosphate (Gpp(NH)p)-, NaF-, D,L-isoproterenol- and glucagon-stimulated adenylate cyclase activities by 18-38% while secretin stimulation was unaffected. In heart membranes from SHR rats, Gpp(NH)p, NaF, D,L-isoproterenol, or glucagon stimulation of the enzyme was similarly increased by 14-38% whilst the low secretin responsiveness which is characteristic of these animals decreased even further (by 24-47%). These results are consistent with: (1) an up regulation of postsynaptic β-adrenergic receptors coupled to adenylate cyclase after degeneration of adrenergic nerves, and (2) a differential response of secretin receptors coupled to adenylate cyclase in the two strains of rats: there was no change in WKY rats and a decreased response in SHR rats. The possible presence and contribution of presynaptic secretin cardiac receptors is considered.

Spontaneously hypertensive rats | Adenylate cyclase | 6-Hydroxydopamine | Isoproterenol
Presynaptic receptors | Secretin | Glucagon | Heart

1. Introduction

We recently described the presence in the rat heart of a secretin-stimulated adenylate cyclase system (Chatelain et al., 1980b). The secretin responsiveness of the enzyme was found to be severely and specifically impaired in spontaneously hypertensive (SHR) rats from the Okamoto strain (Chatelain et al., 1979; Chatelain et al., 1980a), this alteration being concomitant with the development of hypertension (Chatelain et al., 1980a), not prevented by an efficient antihypertensive treatment (Chatelain et al., 1981), and reproduced in normotensive WKY rats by repeated injections of isoproterenol (Chatelain et al., 1982). These findings are consistent with the hypothesis that the characteristic hyperactivity of the norepinephrine pathway of the autonomic system in spontaneously hypertensive rats (Saavedra et al., 1978; Ekas and Lokhandwala, 1981) contributes to the reduced secretin responsiveness of their cardiac adenylate cyclase.

The physiological role(s) of secretin in rat heart and the anatomical localization of secretin receptors coupled to adenylate cyclase are unknown. In the present study, the pre- and/or postsynaptic localization of secretin receptors in the heart of normotensive Wistar Kyoto (WKY) and SHR rats was examined. For this purpose, rats were treated

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TABLE I

Adenylate cyclase activity in cardiac membranes from normotensive (WKY) and spontaneously hypertensive (SHR) rats untreated or treated with 6-hydroxydopamine 2 and 3 weeks before sacrifice. Results were expressed in pmol cyclic AMP formed·min^{-1}·mg protein^{-1} and are the mean ± S.E.M. from 10 animals. The values obtained in the presence of D,L-isoproterenol, glucagon, and secretin were calculated after subtraction of the unstimulated value obtained in the presence of 10^{-5} M GTP.

Rats	Normotensive WKY			Spontaneously hypertensive SHR		
Post treatment period	Controls	Treated		Controls	Treated	
		2 weeks	3 weeks		2 weeks	3 weeks
Basal	36.6 ± 1.8	32.9 ± 2.0	38.0 ± 1.9	28.4 ± 1.7	23.6 ± 1.5	25.8 ± 1.9
Gpp(NH)p 10^{-4} M	152.8 ± 8.3	151.3 ± 7.2	189.5 [a] ± 9.0	93.7 ± 4.3	97.5 ± 5.0	111.5 [a] ± 6.3
NaF 10^{-2} M	398.0 ± 12.0	422.0 ± 18.2	485.0 [a] ± 20.0	279.3 ± 12.0	301.6 ± 16.0	323.9 [a] ± 13.0
GTP 10^{-5} M	42.3 ± 3.0	40.9 ± 2.3	47.4 ± 3.2	32.2 ± 2.0	31.6 ± 1.8	32.8 ± 3.0
GTP 10^{-5} M and D,L-isoproterenol 10^{-4} M	104.2 ± 6.2	122.9 [a] ± 6.1	136.5 [a] ± 8.5	55.6 ± 3.0	71.6 [a] ± 3.5	76.7 [a] ± 4.0
GTP 10^{-5} M and glucagon 10^{-5} M	74.4 ± 3.9	102.7 [a] ± 7.3	93.7 [a] ± 4.8	36.2 ± 3.2	41.3 [a] ± 2.0	47.8 [a] ± 2.5
GTP 10^{-5} M and secretin 3 10^{-5} M	89.8 ± 6.0	96.9 ± 5.3	98.8 ± 4.8	19.9 ± 1.5	10.6 [a] ± 0.9	15.1 ± 1.1

[a] Indicates values significantly different ($P < 0.05$) from the corresponding control values using Student's t-test on unpaired values.

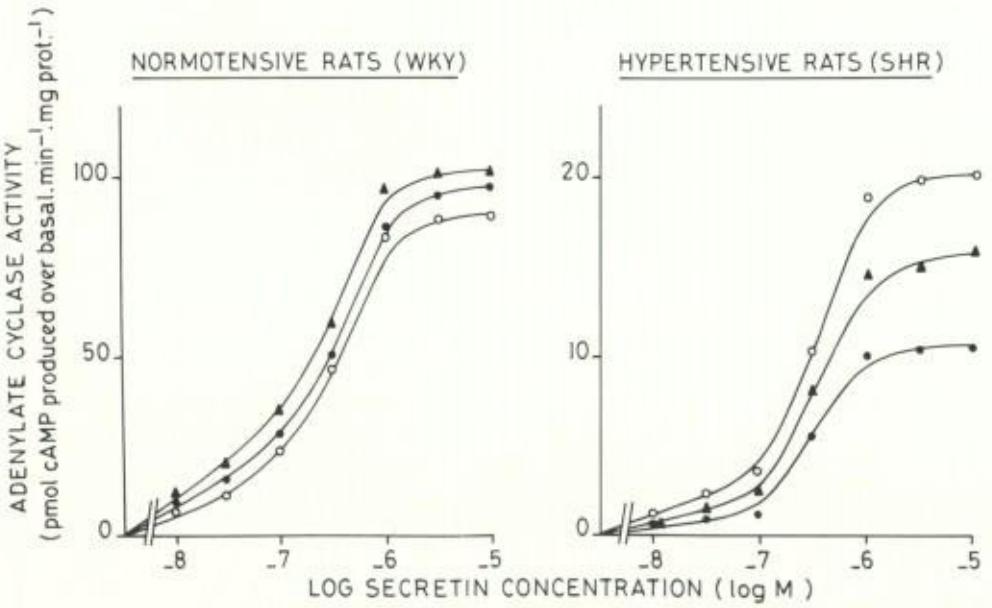

Fig. 1. Dose-effect relationship of secretin on cardiac adenylate cyclase activation of normotensive WKY (*left panel*) and spontaneously hypertensive SHR rats (*right panel*). Heart membranes from control rats (○) were compared to those from rats treated with 6-hydroxydopamine 2 weeks (●) or 3 weeks (▲) before sacrifice. The results, expressed as pmol cyclic AMP produced·min^{-1}·mg protein^{-1} over the basal value, were the means of duplicate determinations on heart membranes from 10 animals.

with 6-hydroxydopamine in order to destroy specifically the catecholaminergic nerve endings (Kostrzewa and Jacobowitz, 1974; Sachs and Jonsson, 1975). The cardiac secretin receptors coupled to adenylate cyclase were evaluated 2 and 3 weeks after the injections. Basal, GTP-, guanosine 5'-O-(2-3-imido)-triphosphate (Gpp(NH)p)-, NaF-, isoproterenol- and glucagon-stimulated adenylate cyclase activities served as reference.

2. Materials and methods

2.1. Animals

The male SHR rats used were derived from the Okamoto strain (CpH_3b) inbred in our laboratory for 10 years and were aged 15 weeks at the beginning of the experiment. Normotensive controls (WKY) were male Wistar/Kyoto rats from the same strain supplied by the Charles River Company (St. Antoine-lez-Elboeuf, France). The animals were injected intravenously (i.v.) with 6-hydroxydopamine (administered in two doses of 50 mg/kg body weight at a 24 h interval) or with the vehicle only. Systolic blood pressure and heart rate of the awake animals were measured before injections and every week thereafter by well trained technicians using a plethysmographic method requiring a pneumatic pulse transducer (MK III, E and M, Houston, TX, U.S.A.), a mercury manometer, and a metallic occluding tail cuff.

The animals were sacrificed 2 or 3 weeks after injections by decapitation and were exsanguinated. The heart was dissected out, quickly rinsed in 0.15 M NaCl at room temperature, weighed and stored in liquid nitrogen.

2.2. Preparation of a particulate fraction from the heart

Cardiac membranes were prepared from individual hearts as previously described (Chatelain et al., 1980b) and stored in liquid nitrogen until use at a protein concentration of 6 mg/ml. Proteins were determined according to Lowry et al. (1951) using bovine serum albumin as a standard.

2.3. Adenylate cyclase assay

Adenylate cyclase activity was determined as described previously (Chatelain et al., 1980b) with minor modifications of the Salomon et al. (1974) procedure.

2.4. Chemicals

Cyclic [8-^{3}H]AMP (specific radioactivity of 24 Ci/mmol) and [α-^{32}P]ATP (specific radioactivity of 20 Ci/mmol) were obtained from the Radiochemical Centre (Amersham, England) and New England Nuclear (Dreieich, F.R.G.), respectively. Synthetic secretin was from Fluka (Bucks, Switzerland); porcine glucagon was a gift from Novo Industri (Ets. Couvreur, Brussels, Belgium). Phospho(enol)pyruvate, pyruvate kinase, cyclic AMP, ATP (sodium salt, grade I), 6-hydroxydopamine, and D,L-isoproterenol were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). GTP and Gpp(NH)p were from Boehringer Mannheim (F.R.G.). All other reagents were of the highest grade available.

3. Results

3.1. Effects of 6-hydroxydopamine treatment on body weight, heart weight, heart rate, and blood pressure in WKY and SHR rats

The systolic blood pressure (204 ± 2 mm Hg, mean $\pm$ S.E.M., $n = 5$) and heart weight (1.22 ± 0.02 g) were significantly higher in untreated SHR rats than in untreated WKY rats (155 ± 1 mm Hg and 0.97 ± 0.03 g, respectively). Two weeks after 6-hydroxydopamine administration a decrease in body weight (-6%), an increase in heart rate ($+6\%$), and an increase in heart body weight ratio ($+7\%$) were observed in SHR rats. These variations were both moderate and transient, i.e. no longer present after 3 weeks. The same parameters were not influenced by 6-hydroxydopamine in WKY rats (data not shown).

3.2. Differences between adenylate cyclase activities in heart membranes from untreated WKY rats and untreated SHR rats

Cardiac adenylate cyclase activities were evaluated in the absence of stimulus, and in the presence of Gpp(NH)p, GTP, NaF, D,L-isoproterenol, glucagon or secretin. The three hormones were tested in the presence of 10^{-5} M GTP. Since there was no change in the values observed in control WKY and SHR rats sacrified 2 and 3 weeks after injection of the vehicle, these data were pooled (table 1 and fig. 1).

Basal-, GTP, Gpp(NH)p- and NaF-stimulated cardiac cyclase activities were reduced by 22, 24, 39 and 30%, respectively, in SHR as compared to WKY rats. Hormone-stimulated enzyme activities were affected even more in SHR rats: by 47, 51 and 78%, respectively, in the presence of D,L-isoproterenol, glucagon, and secretin.

3.3. Effects of 6-hydroxydopamine treatment on adenylate cyclase activity in heart membranes from WKY rats

The 6-hydroxydopamine treatment exerted no effect on basal, GTP-stimulated, and GTP plus secretin-stimulated adenylate cyclase activities. By contrast, Gpp(NH)p-, NaF-, D,L-isoproterenol- and glucagon-stimulated activities increased by 20-40%. Variations in the presence of D,L-isoproterenol and glucagon were already significant 2 weeks after drug administration whereas those observed with Gpp(NH)p and NaF were only significant after 3 weeks (table 1). The dose-effect relationships of secretin, D,L-isoproterenol and glucagon showed no change in the hormone concentration required for half-maximal adenylate cyclase activation (fig. 1, left panel and data not shown).

3.4. Effects of 6-hydroxydopamine treatment on adenylate cyclase activity in heart membranes from SHR rats

Basal and GTP-stimulated adenylate cyclase activities were unaffected by the treatment. D,L-Isoproterenol-stimulated adenylate cyclase activity increased after 2 weeks whereas Gpp(NH)p-, NaF- and glucagon-stimulated activities only increased after 3 weeks. At variance with the data on normotensive WKY rats (see above), secretin stimulation decreased by 47 and 24%, respectively, 2 and 3 weeks after 6-hydroxydopamine administration (table 1). This reduction in secretin-stimulated adenylate cyclase activity was not accompanied by a change in the hormone concentration required for half-maximal enzyme activation (fig. 1).

4. Discussion

Although the norepinephrine content of the myocardium was not measured directly after 6-hydroxydopamine injections, indirect arguments suggest that the treatment was indeed effective in both WKY and SHR rats: (a) our experimental protocol was exactly the same as that utilized by Yamada et al. (1980) who observed a 60-80% reduction in heart norepinephrine concentration after 2 weeks; (b) the higher cardiac adenylate cyclase activity, denoted in both WKY- and SHR-treated rats by 16-24% increases in Gpp(NH)p- and NaF-stimulated enzyme activities (table 1), was of a magnitude comparable to that noted by Chiu (1978) after 6-hydroxydopamine injection and by Palmer et al. (1975) after surgical heart denervation; (c) the present 20-40% increases in isoproterenol-stimulated enzyme activities in WKY- and SHR-treated rats (table 1) were also comparable to those reported by Chiu (1978) and in line with the increased number of β-adrenoceptors observed after 6-hydroxydopamine treatment (Nomura et al., 1980; Yamada et al., 1980). Finally, our experiments were conducted 2 and 3 weeks after the 6-hydroxydopamine injections, a period considered as optimal for changes in hormone receptors following chemical denervation (Yamada et al., 1980).

The two major findings of the present study were: (1) An increased activity of the catalytic unit and/or the guanine nucleotide regulatory site(s) of the cardiac adenylate cyclase system, in response to chemical denervation in both WKY and SHR rats (table 1). A similar adaptation of adenylate

cyclase components located at the inner face of membranes has already been mentioned for the heart (Palmer et al., 1975; Chiu, 1978) but not in the central nervous system following the intracisternal administration of 6-hydroxydopamine (Sporn et al., 1977). The even greater increase in D,L-isoproterenol-stimulated adenylate cyclase activity might reflect an increased number of β-adrenergic receptors i.e. an 'up regulation' due to a reduced catecholamine content (Nomura et al., 1980; Yamada et al., 1980). That the increase in glucagon-stimulated adenylate cyclase activity was of the same order of magnitude as that observed with D,L-isoproterenol is difficult to explain: it is tempting to suggest either an up regulation of glucagon receptors or a better coupling process between glucagon receptors and the catalytic subunit. (2) The effects of 6-hydroxydopamine chemical denervation on secretin-stimulated adenylate cyclase activity differed drastically from those on D,L-isoproterenol- and glucagon-stimulated enzyme activities (table 1). The absence of modification of the secretin responsiveness in WKY rat heart membranes, despite the general increase in adenylate cyclase activity, could reflect a relative decrease in the number of secretin receptors due to the treatment. This hypothesis is better supported by the data obtained with treated SHR rats, because the secretin response was already impaired in untreated hypertensive animals; in these rats, 6-hydroxydopamine induced a further selective reduction in secretin-stimulated adenylate cyclase activity (table 1). If we admit that 6-hydroxydopamine provoked a selective destruction of presynaptic nerve endings of the catecholaminergic pathways in the heart of both WKY and SHR rats (Kostrzewa and Jacobowitz, 1974; Sachs and Jonsson, 1975), these data may be interpreted in terms of the relative abundance of pre- and postsynaptic secretin receptors coupled to their respective adenylate cyclase systems. In the WKY rat heart, a majority of secretin receptors could be located postsynaptically. By contrast, the further decrease in secretin receptors after the selective destruction of nerve endings from catecholamine producing neurons would be rendered possible if there were a larger proportion of pre-synaptic secretin receptors in the untreated SHR rat heart.

This interpretation, strengthened by the finding of peptidergic nerves in the heart of animals from several species (Weihe and Reinecke, 1981), suggests that secretin might act as a neuromodulator in heart nerves, controlling the presynaptic release of catecholamine through adenylate cyclase stimulation (Langer, 1980). Other interpretations, including β-adrenergic control of postsynaptic secretin receptors may also be considered. At any rate, the present data, taken together with previous information (Chatelain et al., 1980a, 1981, 1982), emphasize the close relationship between β-adrenergic nerves and secretin receptors.

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