LOW RESPONSIVENESS OF CARDIAC ADENYLATE CYCLASE ACTIVITY TO PEPTIDE HORMONES IN SPONTANEOUSLY HYPERTENSIVE RATS

P. CHATELAIN, P. ROBBERECHT, P. DE NEEF, M. CLAEYS and J. CHRISTOPHE*

Department of Biochemistry and Nutrition, Medical School, Université Libre de Bruxelles, Boulevard de Waterloo 115, B-1000 Brussels and *Continental Pharma Research Laboratories, B-1830 Machelen, Belgium*

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1. Introduction

Besides the early development of hypertension, the Wistar-Okamoto substrain of spontaneously hypertensive rats (SH) exhibits an impaired cardiac performance characterized by decreased chronotropic and inotropic responses to β-adrenergic stimulation [1,2]. The origin of these alterations in heart mechanical responses may involve primary or compensatory mechanisms. A 30% decrease in the total number of β-adrenergic binding sites was recently described in cardiac membranes from SH rats as compared to normotensive Wistar-Kyoto (WKY) control rats [3]. The affinity of these receptors was found to be identical in both groups of animals [3] despite a lesser sensitivity of adenylate cyclase activity towards catecholamines previously reported in cardiovascular tissues from SH rats [4].

More recently, a decreased glucagon- and fluoride-stimulated adenylate cyclase activity was documented in the heart of SH rats [5]. The efficacy and potency of secretin and VIP, two parent peptides of glucagon which also stimulate cardiac mechanical properties [6–9], is unknown.

This report compares the stimulation of adenylate cyclase activity in stable, highly responsive preparations of cardiac membranes from spontaneously (genetic) hypertensive SH rats, renal hypertensive rats of the Goldblatt type, and their normotensive controls (WKY rats and sham-operated Sprague-Dawley rats, respectively). Three types of stimulants were tested:

(i) Agents acting at the receptor level including the β-adrenergic agonist isoproterenol, and the peptides glucagon, secretin, and vasoactive intestinal peptide;
(ii) GTP and Gpp(NH)p which act at the guanyl regulatory site(s);
(iii) NaF which directly stimulates the catalytical subunit.

A striking reduction in secretin responsiveness was the major finding in cardiac membranes from SH rats. This contrasted with normal properties of the adenylate cyclase activity in similar preparations from renal hypertensive rats.

Abbreviations: VIP, vasoactive intestinal peptide; Gpp(NH)p, guanosine 5'-(β,γ-imido)triphosphate

* To whom correspondence should be addressed

2. Materials and methods

All strains of rats were bred locally. The main characteristics of the male animals used are summarized in table 1. Goldblatt animals were prepared by a stricture on the right renal artery 6 weeks before sacrifice. The left kidney was left untouched. The corresponding controls were sham-operated.

After an overnight fast the rats were sacrificed by decapitation. Each heart was carefully dissected out, rinced at room temperature with isotonic sodium chloride, weighed, and frozen in liquid nitrogen.

A crude cardiac particulate fraction was prepared with minor modifications of the procedure in [10]. The cardiac tissue was homogenized (5%, w/v, homogenate) in a 20 mM Tris–HCl, 2 mM dithio-

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erythreitol, 5 mM $MgCl_2$ (pH 7.5) buffer. After filtration through two layers of medical gauze the homogenate was centrifuged at 520 × *g* for 10 min. The pellet was washed once with the same buffer and resuspended in 20 mM Tris–HCl, 5 mM $MgCl_2$, 0.25 M sucrose (pH 7.5) buffer. An equal volume of the same buffer enriched with 2.5 M KCl was added dropwise. The suspension was stirred continuously for 2 h at 2°C then centrifuged at 37 000 × *g* for 10 min. The pellet was resuspended in a 20 mM Tris–HCl, 2 mM dithioerythreitol, 0.25 M sucrose (pH 7.5) buffer, washed 3 times in this buffer by centrifugation at 31 000 × *g* for 5 min and resuspended in a volume of the same buffer allowing final conc. 6 mg/protein ml.

Adenylate cyclase activity was determined with minor modifications of the procedure in [11]: ~60 μg membrane protein were incubated in 60 μl containing 0.5 mM [α-^{32}P]ATP, 5 mM $MgCl_2$, 0.5 mM EGTA, 1 mM cyclic AMP, 1 mM theophylline, 10 mM phospho(enol)pyruvate, 30 μg/ml pyruvate kinase and 30 mM Tris–HCl, adjusted to pH 7.5. Under all conditions tested, the enzymatic kinetics were linear for ⩾ 10 min at 37°C. In routine assays, the reaction was stopped after 10 min by adding 0.5 ml of a 0.5% sodium dodecylsulfate solution containing 1.5 mM ATP, 0.5 mM cyclic AMP and 20 000 cpm cyclic [8-^{3}H]AMP. Cyclic AMP was separated from ATP by two successive chromatographies on Dowex AGI × 8 and neutral alumina.

DL-isoproterenol was from Sigma Co. (St Louis, MO) and porcine glucagon was from Novo Industri (Copenhagen). Natural porcine secretin and VIP were essentially pure peptides prepared by Dr Viktor Mutt at the Karolinska Institutet (Stockholm) and were supplied by the NIH (Bethesda, MD).

3. Results

Basal, fluoride-, Gpp(NH)p- and GTP-activated adenylate cyclase activities were determined in the standard medium assay in section 2. The hormone-sensitive activity was measured in the presence of 10^{-5} M GTP as orientation experiments indicated that neither a β-adrenergic agonist, nor glucagon, secretin or VIP could activate the enzyme in the absence of a guanyl triphosphate nucleotide.

All the agents tested proved to be effective stimuli of cardiac adenylate cyclase activity (fig. 1 and table 1).

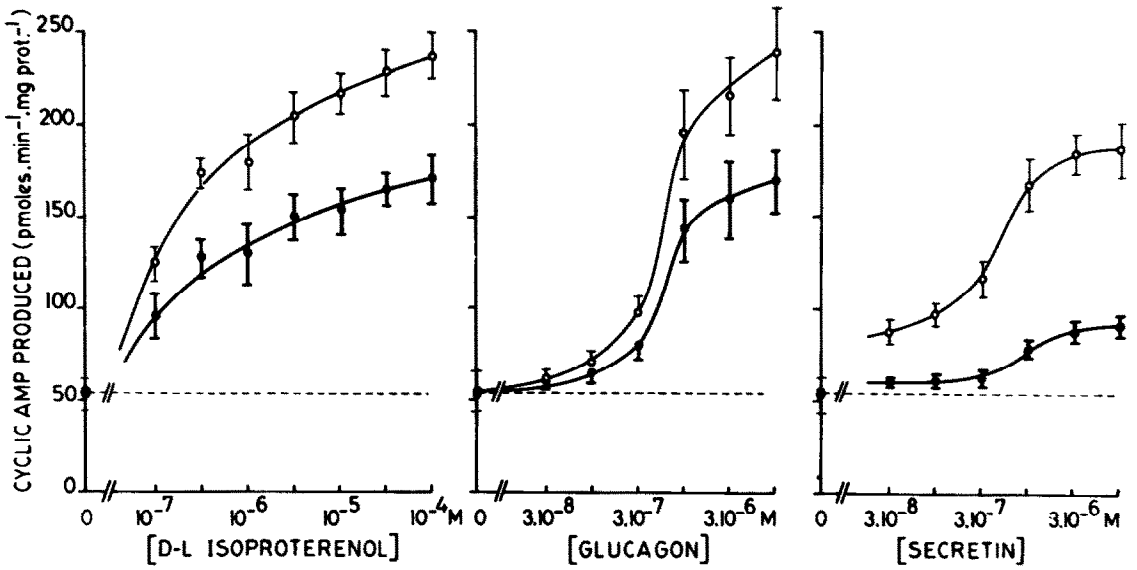

Fig.1. Dose–effect curves of adenylate cyclase activity stimulated by isoproterenol (left panel), glucagon (middle panel) and secretin (right panel), in the presence of 10^{-5} M GTP, in cardiac membranes from WKY rats (white circles) and SH rats (black circles). Each curve is the mean ± SEM of 5 expt. The dashed lines indicate that values obtained in the presence of 10^{-5} M GTP alone.

Table 1
General characteristics of types of male hypertensive rats and their corresponding controls

Group	WKY rats	SH rats	Sham-operated	Goldblatt rats
Strain	Wistar	Wistar	Sprague-Dawley	Sprague-Dawley
Substrain	Kyoto(WKY)	SH	–	–
Age (weeks)	14	14	18	18
Body weight (in g)	273 ± 4	272 ± 12	395 ± 9	377 ± 14
Systolic blood pressure (in mm Hg)	132 ± 5	200 ± 6[a]	107 ± 4	183 ± 8[a]
Heart weight (in mg)	830 ± 20	920 ± 20	922 ± 45[a]	1164 ± 44[a]

[a] Significantly higher (at $P < 0.05$) than values obtained with corresponding normotensive controls by the Student's t-test

Means ± SEM from 5 animals

There was not significant difference in the magnitude of the stimulations observed with 10^{-5} M GTP, 10^{-5} M Gpp(NH)p and 10 mM NaF in heart membranes from normal and hypertensive animals of both the Wistar-Kyoto and the Sprague-Dawley strains. Dose–effect curves of adenylate cyclase activity stimulated by isoproterenol, glucagon and secretin indicate that the 3 agents were potent activators in the presence of 10^{-5} M GTP. However, maximal effects of isoproterenol, glucagon and secretin were lower in membranes from SH rats than in control WKY rats with no modification in the concentration required for half-maximal efficacy. The stimulatory effect exerted by 3×10^{-6} M VIP was also significantly lower in SH rats as compared to WKY rats (table 1). The secretin responsiveness was by far the most affected (fig. 1 and table 1) being statistically lower ($P < 0.05$) than that observed with the other stimulatory agents.

In renal hypertensive Goldblatt rats on the contrary, the maximal stimulatory effects of isoproterenol, glucagon and secretin were identical with those observed in sham-operated rats of the Sprague-Dawley strain (table 2). The amine and peptide concentrations

Table 2
Stimulation of adenylate cyclase activity in cardiac membranes from spontaneously hypertensive (SH) and Goldblatt renal hypertensive rats and their corresponding controls

Additions	(M)	WKY rats	SH rats	Sham-operated	Goldblatt rats
GTP	10^{-5}	8 ± 2	8 ± 2	5 ± 1	4 ± 1
Gpp(NH)p	10^{-5}	100 ± 15	87 ± 10	149 ± 18	107 ± 24
NaF	10^{-2}	503 ± 60	470 ± 33	610 ± 22	560 ± 20
DL-Isoproterenol	10^{-4}	187 ± 15	123 ± 11[a]	104 ± 18	93 ± 15
Glucagon	10^{-5}	189 ± 25	114 ± 15[a]	86 ± 12	93 ± 13
Secretin	10^{-5}	133 ± 11	37 ± 5[a]	111 ± 12	97 ± 15
VIP	3×10^{-6}	46 ± 5	20 ± 4[a]	50 ± 9	48 ± 8

[a] Significantly lower (at $P < 0.05$) than values obtained with corresponding normotensive controls, by the Student's t-test

The data are expressed as pmol cyclic AMP formed .min^{-1} .mg protein^{-1} and were obtained after subtracting the basal unstimulated value. Means ± SEM from 5 animals. The mean basal activities were 47 ± 6, 43 ± 6, 62 ± 7 and 48 ± 5 in cardiac membranes from WKY, SH, sham-operated and Goldblatt rats, respectively

required for half-maximal efficacy were also equivalent in both groups and similar to those observed in the Wistar-Kyoto strain (data not shown). In addition, noticeable differences in the maximal efficacy of stimuli were observed between preparations from control normotensive rats of the Wistar-Kyoto and Sprague-Dawley strains (table 2).

4. Discussion

The present data demonstrate that:

1. In two different strains, rat cardiac adenylate cyclase activity was GTP-dependently stimulated by secretin and VIP as well as by the two well established stimulatory agents isoproterenol and glucagon;
2. Peptide- and isoproterenol-stimulated adenylate cyclase activities were lowered in adult SH rats with no apparent alteration in either guanyl nucleotide regulatory sites or fluoride-sensitive sites. The reduction in maximal responses to the hormone peptides and to the β-adrenergic agonist, with no change in their efficacy, suggests a decrease in the number of the corresponding receptors. In this respect, the 34% lowering of maximal isoproterenol binding sites of cardiac membranes reported [3]; stimulation of adenylate cyclase activity in SH rats (table 1) compared well with the 30% reduction in dihydroalprenolol binding sites of cardiac membranes reported [3];
3. The secretin-stimulated adenylate cyclase activity was more reduced than that observed in the presence of any other agent;
4. Taken together, these results suggest that secretin, VIP and glucagon functional receptors represented distinct entities, in spite of the numerous structural homologies between the 3 peptides [12]. This situation is reminiscent of the existence of distinct glucagon and secretin–VIP receptors in liver [13] and distinct secretin- and VIP-preferring receptors in the exocrine pancreas [14];
5. The reduced hormone sensitivity of adenylate cyclase appeared specific to the spontaneously hypertensive rat as the same system was free of such alterations in animals with renal hypertension exhibiting similar high systolic blood pressure and cardiac muscle hypertrophy. Thus, impairment of cardiac hormone-sensitive adenylate cyclase activity appeared to be part of the syndrome in SH rats and not a consequence of myocardial strain.

The preferential reduction in the potency of secretin and VIP as activators of adenylate cyclase in cardiac membranes from SH rats raises the dual question of its bearing on cardiac muscle activity and on the role of secretin and VIP in the pathogenesis of the syndrome. Data concerning the effects of secretin and VIP on heart metabolism are scarce: both peptides share with glucagon and isoproterenol stimulating properties on cardiac activity [6–9] but the physiological significance of these effects remains to be established. In addition, the regulation of hormone receptors in heart is largely unknown, e.g., the adaptation of the number of receptors in response to alteration in the level of circulating hormone or in the activity of sympathetic innervation. A few lines of evidence allow, however, some speculation on a possible involvement of secretin and VIP in the biochemical events leading to hypertension in SH rats. The 3 prevalent hypotheses accounting for the development of hypertension in SH rats might indeed imply a contribution of these peptides:

1. The capacity of the kidney to excrete salt and water in proper relation to their intake is reduced [15]. Secretin and VIP are known to influence several water movements. Secretin inhibits the reabsorption of H^+ and Na^+ in the human kidney [16]. Secretin and VIP stimulate bicarbonate and water secretion from the exocrine pancreas [17]. VIP stimulates fluid secretion from gut [18]. VIP is present in large amounts in the hypothalamus [19] and also in the posterior pituitary [20] where it activates a particulate adenylate cyclase [21]. This might provide a link between VIP and vasopressin, renin, angiotensin 2, and ACTH secretion [22].
2. SH rats suffer from an hyperactivity of the sympathoadrenal system [23]. The candidate neurotransmitter VIP could modulate neuronal transmission in both central and sympathetic nervous systems [24,25]. VIP is also reported to activate adenylate cyclase in adrenal glands [26]. A role for secretin in the nervous system is not yet documented.
3. There is an enhanced vasoconstrictor response of

vascular smooth muscle in SH rats. VIP is a potent vasodilator [27] which probably acts through the rich VIPergic innervation of blood vessels. Similarly, secretin provokes a vasodilatation of splanchnic vessels [28].

From our data and from the latter considerations, it is tempting to suggest that a low responsiveness of adenylate cyclase to secretin and VIP could alter the mechanical performance of the myocardium in SH rats and that this class of peptides might be involved in the pathogenesis of hypertension in these animals.

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