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Effects of a chemical sympathectomy on cardiac muscarinic receptors in normotensive (WKY) and spontaneously hypertensive (SHR) rats

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The rate and contractility of the heart are regulated by the parasympathetic system through muscarinic receptors. Muscarinic receptors tested in binding studies with a labelled antagonist behave as a homogeneous class of receptors but can be distinguished into subclasses with respect to their affinity for agonist molecules [1]. Yamada *et al.* [2] suggested that, at least in rat, most of these cardiac muscarinic cholinergic receptors are located postsynaptically and are not related to adrenergic neurons. Sharma and Banerjee [3] have concluded, however, that a large number of these muscarinic receptors are located presynaptically on noradrenergic nerve endings, based on the observation that the number of these receptors decreases markedly following 6-OH dopamine* administration, a treatment that selectively destroys nerve endings taking up catecholamines [4]. This interpretation is supported by the pharmacological demonstration of a reduction, by muscarinic agonists, of the stimulation-evoked release of norepinephrine in heart [5, 6].

Spontaneously hypertensive (SHR) rats from the Okamoto strain exhibit hyperactivity of the norepinephrine pathway of the autonomic system [7, 8] that leads to a decrease in the number of β-adrenergic receptors in the heart [9–11]. In the same tissue, the total number of muscarinic receptors is not modified [11] but the relative proportion of high- and low-affinity binding sites for agonists, as well as the balance of pre- and postsynaptic muscarinic receptors, has not yet been documented.

We recently described a methodological approach [12, 13] for the determination in heart membranes of the relative densities of high- and low-affinity binding sites for muscarinic agonists based on the use of two radioactive ligands: the antagonist L-[^{3}H]QNB and the agonist [^{3}H]oxo-M. In the present study, we used this method to compare the distribution of muscarinic cholinergic receptor subclasses in the heart from normotensive (WKY) and spontaneously hypertensive (SHR) rats before and after chemical sympathectomy with 6-OH dopamine.

Experiments were conducted on male SHR rats of Okamoto strain, 15 weeks of age at the beginning of the experiment, and age-matched with normotensive Wistar–Kyoto (WKY) rats. Animals were injected intravenously with 6-OH dopamine, administered in two doses of 50 mg/kg body weight at a 24-hr interval. Control animals received an equivalent volume of the vehicle. The rats were sacrificed 3 weeks after the first injection. Each heart was dissected out, rinsed with 0.15 M NaCl, weighed and stored in liquid nitrogen until use.

The schedule utilized for 6-OH dopamine injections was exactly that proposed by Yamada *et al.* [2]. The norepinephrine content of heart was not measured in the present study but the efficacy of the treatment was indirectly demonstrated by increased 10^{-4} M D,L-isoproterenol-stimulated adenylate cyclase activity in heart membranes (+30% in WKY rats and +45% in SHR rats, on average).

Membranes were prepared from thawed hearts by homogenization (5%, w/v homogenate) at 4° in a buffer consisting of 20 mM Tris–HCl (pH 7.4), 2 mM dithioerythritol and 5 mM $MgCl_2$. After filtration through two layers of medical gauze, the homogenate was centrifuged at 520 *g* for 10 min. The crude particulate extract was treated as previously described [14] and membranes obtained were tested for muscarinic receptors.

Heart membrane proteins (90–110 μg) were incubated for 30 min at 25° with increasing concentrations of L-[^{3}H]QNB (specific radioactivity 40 Ci/mmole) or [^{3}H]oxo-M (specific radioactivity 84 Ci/mmole), obtained from New England Nuclear Corporation (Dreieich, FRG) in 1.2 ml of 50 mM sodium phosphate buffer (pH 7.5) enriched with 1 mM $MgCl_2$, and in the absence or presence of 1 μM atropine (in order to determine the non-specific binding). Membrane-bound radioactivity was separated from free radioactivity by filtration through glass-fibre filters GF/C (Whatman, Maidstone, U.K.) and washed three times with ice-cold buffer. The methodology used has been previously detailed [12, 13]. Under these experimental conditions, L-[^{3}H]ONB bound to all muscarinic receptors whereas [^{3}H]oxo-M bound only to high-affinity receptors for muscarinic agonists.

The main characteristics of the animals are detailed in Table 1. 6-OH dopamine treatment was without effect on body weight increase, heart rate and blood pressure. The cardiac hypertrophy of SHR rats was also not altered by the drug.

The total number of muscarinic sites measured by L-[^{3}H]QNB binding, the proportion of high-affinity and low-affinity sites for the agonist [^{3}H]oxo-M, and the dissociation constants (K_D) of the receptors for both ligands were identical in normotensive (WKY) and hypertensive (SHR) rats (Table 2 and Fig. 1).

In WKY rats, neither the total number of muscarinic receptors nor the number of high-affinity agonist binding sites was modified following 6-OH dopamine treatment (Table 2 and Fig. 1, left panel). These negative data are in line with those of Story *et al.* [15] and Yamada *et al.* [2] (the latter group reported a significant increase in muscarinic receptor density only in the atria and not in the ventricles).

In SHR rats, the total number of muscarinic receptors was unaltered by 6-OH dopamine treatment but the density of the high-affinity binding sites for agonists showed a 63% increase. The number of low-affinity binding sites for agonist (i.e. the total number of receptor minus the number

* Abbreviations used: [^{3}H]oxo-M, [methyl-^{3}H]oxotremorine acetate; L-[^{3}H]QNB, L-[benzilic-4,4′-^{3}H]quinuclidinylbenzilate; 6-OH dopamine, 6-hydroxydopamine.

Table 1. General characteristics of normotensive (WKY) and spontaneously hypertensive (SHR) rats before and 3 weeks after a 6-OH dopamine treatment

Parameter		Normotensive (WKY) rats		Hypertensive (SHR) rats	
		Control	6-OH Dopamine	Control	6-OH Dopamine
Body weight (g)	Initial	281 ± 4	283 ± 5	275 ± 7	279 ± 7
	Final	301 ± 3	302 ± 6	288 ± 5*	289 ± 8
Systolic blood	Initial	146 ± 2	150 ± 3	199 ± 5*	199 ± 5*
Pressure (mm Hg)	Final	156 ± 2	155 ± 2	209 ± 5*	201 ± 4*
Heart rate	Initial	324 ± 14	309 ± 14	318 ± 9	321 ± 8
(beats/min)	Final	324 ± 9	339 ± 16	330 ± 9	327 ± 9
Heart weight (g)	Final	1.02 ± 0.15	1.00 ± 0.10	1.21 ± 0.10	1.22 ± 0.09
Heart weight/ body weight × 100		3.39 ± 0.02	3.31 ± 0.02	4.20 ± 0.03*	4.22 ± 0.02*

The results are the means ± S.E.M. from five animals. The treatment was without significant effect in both normotensive and hypertensive rats.

* Values significantly different ($P < 0.05$) in SHR rats as compared to WKY rats using Student's t-test on unpaired values.

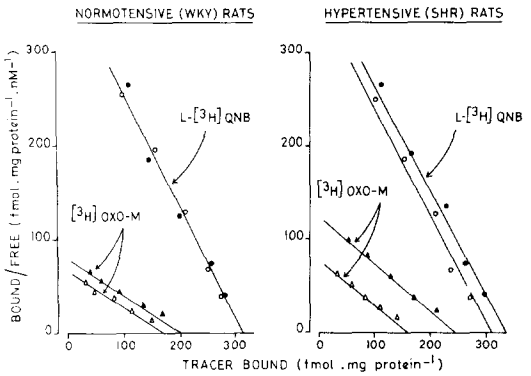

Fig. 1. Scatchard analysis of L-[^{3}H]QNB (○,●) and [^{3}H]oxo-M-M (△, ▲) binding to cardiac membranes from normotensive WKY (left panel) and spontaneously hypertensive SHR (right panel) rats untreated (open symbols) or treated (closed symbols) with 6-OH dopamine 3 weeks before sacrifice. The binding assays were performed according to the standard procedure described in [12]. Each point represents the mean of duplicate determinations from five animals.

of high-affinity receptors) was reduced by half (Table 2 and Fig. 1, right panel). The dissociation constants of the tracers were not affected by the drug administration.

It is usually considered that a 6-OH dopamine treatment selectively destroys the nerve endings of catecholamine producing neurons and that, as a consequence, any decrease in the number of neuroreceptors of a given type after this chemical lesion is rendered possible by their previous presence on these nerve endings, whilst a status quo or an increased number of these receptors (denervation supersensitivity) reflects their postsynaptic nature. If this is true, and if the two muscarinic receptor subclasses considered here represent distinct entities, the low-affinity and high-affinity binding sites for muscarinic agonists must then be located pre- and post-synaptically, respectively, in the heart of SHR rats. On the other hand, if the two classes of heart muscarinic receptors correspond to one entity existing in two functional states, the present results could be interpreted as follows: muscarinic receptors were essentially located postsynaptically, their number was unaffected by the 6-OH dopamine treatment but the chemical sympathectomy induced a conversion from the low-affinity to the high-affinity state for agonist. Whatever the exact underlying mechanism, the response in SHR and WKY rat heart to the same administration schedule of the neurotoxic differed obviously. This might reflect differences in localiza-

Table 2. Effects of 6-OH dopamine treatment on the dissociation constants (K_D) and receptor density (B_{max}) for L-[^{3}H]QNB and [^{3}H]oxo-M in rat cardiac membranes from normotensive (WKY) and hypertensive (SHR) rats

		L-[^{3}H]QNB		[^{3}H]Oxo-M	
Rat	Treatment	K_D (nM)	B_{max} (fmole/ mg protein)	K_D (nM)	B_{max} (fmole/ mg protein)
WKY	0	0.65 ± 0.07	312 ± 20	2.5 ± 0.3	172 ± 15
	+	0.65 ± 0.08	312 ± 28	2.5 ± 0.3	193 ± 12
SHR	0	0.74 ± 0.08	303 ± 25	2.0 ± 0.3	150 ± 13
	+	0.80 ± 0.09	322 ± 30	2.0 ± 0.4	245 ± 15*

The K_D and B_{max} values were the means (± S.E.M.) from five animals and were derived from Scatchard analysis.

* Value significantly higher ($P < 0.025$) than the corresponding control value using Student's t-test on unpaired data.

tion and/or control mechanisms of muscarinic receptors or a distinct susceptibility of the two strains of rat to 6-OH dopamine (the drug efficacy can, indeed, be modified by the circulatory flow and by the importance of catecholamine uptake in nerve endings [16], two parameters that might also differ in SHR and WKY rats).

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Department of Biochemistry and Nutrition
Medical School
Université Libre de Bruxelles
Bld de Waterloo 115
B-1000 Brussels
Belgium
MAGALI WAELBROECK
PATRICK ROBBERECHT
JEAN CHRISTOPHE*

Continental Pharma Research Laboratories
B-1348 Mont-Saint-Guibert
Belgium
PIERRE CHATELAIN
ANH NGUYEN HUU
JOSEPH ROBA

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* To whom correspondence should be addressed.

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Effects of antilipolytic agents on peroxisomal β-oxidation of fatty acids in rat liver

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Peroxisomes can perform β-oxidation of fatty acids [1]. There are now many reports on the induction of this oxiditive activity in the liver after the administration of hypolipidemic drugs (see e.g. [2]) or under conditions of sustained high hepatic influx of fatty acids (see e.g. [3, 4]). In this paper we report on the effects of two different antilipolytic drugs on this activity in comparison with the effects of the natural antilipolytic agent glucose.

Materials and methods

Experiments were performed on random groups of conventional male Sprague–Dawley rats (190–220 g body weight) housed at 18–25° in plastic cages with a drinking bottle and fed a Randoin–Causeret diet (Piccioni, Brescia, Italy) containing 3.6% lipid. 3,5-Dimethylpyrazole (DMP) was given by intraperitoneal injection of 12 mg/kg (in 0.2 ml saline) every 3 hr [5]. ACIPIMOX (5-methylpyrazine carboxilic acid 4-oxide)—a new antilipolytic drug [6, 7] marketed by Carlo Erba S.p.A. (Milano, Italy)(25 mg/kg body weight, in 1.0 ml saline, pH 2.13) was given intraperitoneally every 3 hr. In both instances, dosages had been found to be maximally active on peroxisomal β-oxidative activity (see also [8]). Glucose (3 g/kg body wt) was administered by stomach tube as a 40% solution (w/v) in water every hour. Controls were given only the vehicles.

After a fasting period of 12 hr, the animals were rapidly bled under nembutal anaesthesia (50 mg/kg body weight) by cutting the femoral vessels, and the blood was collected and then centrifuged. The livers were rapidly removed and weighed. The right lobe was homogenized (1:10, w/v) in 0.25 M sucrose with a Teflon-pestle glass-vessel homogenizer. The peroxisomal β-oxidative activity was measured according to Inestrosa *et al.* [9]. In the normal rat this procedure also assays the acyl-CoA oxidase activity. The homogenate (0.2 ml) was added to 2.8 ml of assay mixture. Final concentrations were: 100 mM Tris–Cl pH 8.3; 100 mM methanol; 1.0 mM Na palmitate (added as a methanol solution); 0.1 mM Coa; 2.5 mM ATP; 5 mM $MgCl_2$; 33 mM nicotinamide; 6.6 mM semicarbazide; 0.2 mM NAD. After 5, 10, 15 and 20 min, aliquots of the assay mixture were transferred into centrifuge tubes containing ice-cold 5% TCA. After centrifugation, formaldehyde was detected in the supernatant according to Nash [10]. Results are given as μmole of formaldehyde/min produced by the liver corresponding to 100 g body wt.

Plasma glucose was assayed by the glucose-oxidase/peroxidase method using commercially available kits (Glucosio Test SCLAVO, Siena, Italy). The levels of plasma FFA and triglycerides were assayed according to [11] and [12], respectively. Liver glycogen was purified and determined spectrophotometrically according to [13]. Liver triglycerides were extracted according to [14] and assayed by a fully enzymatic procedure [12].

All products used were of analytical grade.

Results and discussion

Table 1 shows the changes in the peroxisomal β-oxiditive activity in the liver homogenate after the administration of antilipolytic agents at a maximally active dosage form an appropriate time (see [8]). Data have been given in absolute terms (i.e. as enzyme units in the liver wet weight corresponding to 100 g body weight) to compensate for the