Background fluorescence was obtained by measuring tissue fluorescence in the region of the anterior commissure and the ventromedial nucleus which showed no catecholamine fluorescence. Then, net DA fluorescence was obtained by subtraction of background fluorescence. Results were analysed by Student's t-test.

*Results and discussion.* After treatment with α-MT alone, the DA fluorescence intensities in the CP, ACB and LPZ were 57.16±3.00%, 52.68±2.26% and 77.86±5.05% of control, respectively. The fluorescence reductions of these regions were significant (CP: $p < 0.001$, ACB: $p < 0.001$ and LPZ: $p < 0.01$).

The results after treatment with neuroleptics are shown in the figure. In ACB, which is innervated by the mesolimbic DA system, the acceleration of DA fluorescence disappearance was identical in sulpiride and haloperidol treated rats. However, in CP, which is innervated by the nigro-striatal DA system, the fluorescence intensities in sulpiride- and haloperidol-treated rats were 74.99±6.35% and 39.16±3.45% of the control values, respectively, and the difference between them was significant ($p < 0.01$). Thus, 2 mg/kg of haloperidol accelerated the DA turnover more than 200 mg/kg of sulpiride in the nigrostriatal DA system. In addition, DA fluorescence disappearance after sulpiride was significantly greater in ACB than in CP ($p < 0.05$). Accordingly, sulpiride increased the DA turnover preferentially in the mesolimbic system. On the other hand, haloperidol had a similar effect on both nigro-striatal and mesolimbic DA turnover. In LPZ, which is the terminal region of the tubero-infundibular DA system, no reduction of the fluorescence was observed under these conditions.

The present histochemical results support the previous biochemical findings and suggest that an acute treatment with neuroleptic agents would have little effect on the DA turnover in the median eminence of the rats.

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## Decrease in [$^3$H]ouabain binding sites in heart and brain from spontaneously hypertensive rats

R. Gheyouche, A. Uzan, G. Le Fur and M. Corgier

*Pharmacie Centrale Algérienne, 2, rue Bichat, Alger (Algérie), and Pharmindustrie, Groupe Pharmuka, 35, quai du Moulin de Cage, F-92231 Gennevilliers (France), 1 September 1980*

*Summary.* A decrease in the number of binding sites ($B_{max}$) for [$^3$H]ouabain was observed in the heart (37%) and the brain (22%) of spontaneously hypertensive rats (SHR) when compared with age-matched control Wistar Kyoto rats. No variation was detected in the affinity constant ($K_D$).

In several types of experimental volume expanded hypertension (reno-prival, renal insufficiency, mineralocorticoid hypertensions), the sodium potassium pump activity, estimated through the $Mg^{++}$ dependent $Na^+$, $K^+$ ATPase (E.C. 3.6.1.3), is decreased in arteries, veins and myocardium (for reviews see Haddy et al.[1], Overbeck[2]). In spontaneously hypertensive rats (SHR) whose genetic hypertension could be of central origin[3] the specific activity of brain ATPase was high; so we compared the $Na^+$, $K^+$ ATPase of the Okamoto Aoki strain of SHR to their normotensive controls (Wistar Kyoto rats, WKY) by measuring the [$^3$H]ouabain binding to brain and heart preparations from the 2 consanguineous strains. This technical choice is supported by the fact that there is a specific and stoichiometric ATP dependent binding in brain and heart rat preparations[4,5].

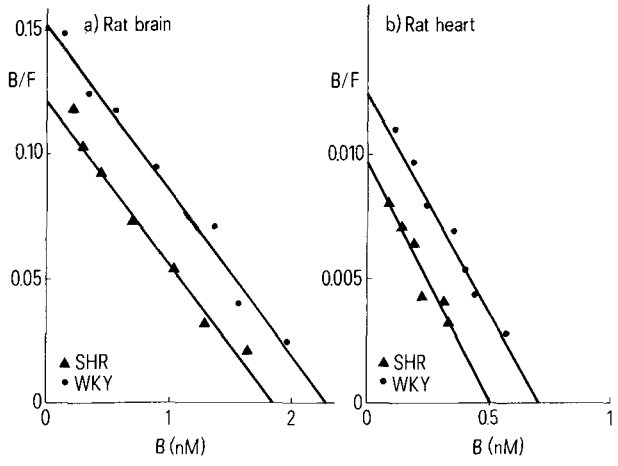
[$^3$H]ouabain (19.3 Ci/mmole) was purchased from New England Nuclear. Male 16-week-old WKY and SH rats, in which hypertension is established, were obtained from Charles River Co. After decapitation brains and hearts were homogenized in 0.32 M sucrose (1:10 w/v and 1:7 w/v respectively). The 49,000×g pellet was diluted in 50 mM Tris HCl buffer pH 7.5 containing 250 mM NaCl, 4 mM $MgCl_2$, 4 mM ATP for brain (40 µg/ml protein) or 20 mM Tris HCl buffer pH 8 containing 150 mM NaCl, 4 mM $MgCl_2$, 4 mM ATP for heart (1 mg/ml protein) and incubated with [$^3$H]ouabain. The reactions were stopped after they had reached their maximum, i.e. 60 min for brain

Binding of [$^3$H]ouabain to brain and heart membranes of spontaneously hypertensive (SH) and normotensive Wistar Kyoto (WKY) rats

| | Brain | | Heart | |
|---|---|---|---|---|
| | $K_D$ (nM) | $B_{max}$ (pmoles/mg protein) | $K_D$ (nM) | $B_{max}$ (pmoles/mg protein) |
| SHR | 14.2±0.4 | 44.6±1.9* | 57.2±9.2 | 0.455±0.036** |
| WKY | 14 ±0.3 | 57.1±3.3 | 50.5±5.4 | 0.722±0.068 |

$K_D$ and $B_{max}$ were determined from Scatchard analysis. Each value is expressed as the mean±SEM of 5 experiments. Each curve is determined by using 7 [$^3$H]ouabain concentrations from 1 to 80 nM for the brain and from 10 to 200 nM for the heart. The rats were 16 weeks old. Systolic blood pressure is 212±6 mm Hg for SHR and 138±3 mm Hg for WKY rats. *$p < 0.02$; **$p < 0.01$.

and 10 min for heart. Specific binding is defined as the difference between total binding and nonspecific binding which is measured in the presence of an excess of cold ouabain (0.1 mM). After filtration the radioactivity trapped on the filters (GF/C Whatman) was determined.




Typical Scatchard plots of ouabain binding in SHR and WKY rat brains and hearts. B, specifically bound [$^3$H]ouabain; F, free [$^3$H]ouabain. SHR brain: 38.1 µg protein/ml; WKY rat brain: 38.7 µg protein/ml; SHR and WKY rat hearts: 1 mg protein/ml.

The affinity constant $K_D$ and the maximal number of sites $B_{max}$ were determined by Scatchard analysis (figure). No difference between the $K_D$ values of SH and WKY rats were observed either in brain or in heart. On the contrary a significant decrease of $B_{max}$ occurred in both organs from SH compared to WKY rats, more pronounced in the heart (37%) that in the brain (22%) (table).

Such a reduced number of binding sites, observed centrally as well as peripherally (heart), is consistent with a decreased $Na^+$, $K^+$ ATPase activity and consequently with a decrease in the $Na^+$ pump, the activity of which extrudes intracellular $Na^+$ and introduces $K^+$.

In experimental volume expanded hypertension the decrease of the $Na^+$, $K^+$ pump does not seem to be a consequence of elevated pressure, since it also occurred in the veins and the right ventricle, where the pressure is not elevated[1]. Whether it is the same in the other models of hypertension, and what the origin of the decrease in ouabain binding sites in SHR is, remain open questions.

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## 3-Hydroxy-4-methoxyphenethylamine, the cardioactive constituent of a soft coral

R.P. Gregson[1], R.R. Lohr, J.F. Marwood and R.J. Quinn

*Roche Research Institute of Marine Pharmacology, P.O. Box 255, Dee Why, N.S.W. 2099 (Australia), 7 May 1980*

*Summary.* Intravenous administration to rats of the aqueous extract of the soft coral *Nephthea* sp. caused an increase in heart rate and blood pressure. The cardioactive constituent was isolated and shown to be 3-hydroxy-4-methoxyphenethylamine.

Routine bioassays of aqueous extracts of marine organisms revealed that the aqueous extract **A** of a soft coral *Nephthea* sp[2] (Coelenterata: Octocorallia) increased heart rate and blood pressure when administered i.v. to DOCA-salt hypertensive, pentobarbitone-sodium anaesthetised rats. Histamine, tyramine, dopamine and/or octopamine were detected in **A** by mass spectrometric selective ion monitoring (SIM) under high resolution conditions[3], but the cardiovascular effects of the extract were not attributable to these biogenic amines. Isolation of the active constituent was achieved by monitoring the fractionation of **A** for tachycardia in hypertensive rats.

An aqueous solution of **A** (200 g, 2.4 l) was diafiltered with a Millipore Pellicon Cell containing 2 membranes having a nominal mol. wt limit of 1000. Lyophilisation of the diafiltrate gave **B** (130 g) which was dissolved in water (1.5 l) and passed through a column (47 × 5 cm) of Amberlite XAD-2 resin. Elution of the resin with methanol (2.5 l) afforded an active fraction **C** (2.2 g) which was chromatographed on Sephadex G-25 (fine) in water.

Only activity attributable to the biogenic amines observed in **A** by SIM was detected in the eluate of the Sephadex column. Therefore, the column was eluted with acetic acid (1.7 M) and a fraction **D** (93 mg) was obtained which displayed tachycardia. Chromatography of **D** on Sephadex G-15 in acetic acid (1.7 M) gave **E** (67 mg) at $V_R/V_M$ = 1.89–2.66, then **E** was subjected to HPLC on a Merck Lichrosorb RP-8 column (4.6 × 250 mm) and the active component **F** (21 mg) eluted with methanol:water (1:1). Preparative thin layer (0.25 mm) chromatography (TLC) of **F** on silica gel (n-butanol:water:acetic acid; 3:1:1) gave **1** (2.3 mg).

Electron impact (e.i.) high resolution mass spectrometry (HRMS) of **1** showed $M^+$ 167 ($C_9H_{13}NO_2$) and major fragments at m/e 138 ($M^+ - CHNH_2$, base peak), 137 ($M^+ - CH_2NH_2$) and 123 ($M^+ - CH_2CH_2NH_2$). The chemical ionisation (isobutane) spectrum afforded a molecular ion at 168 (base peak) and in addition to the fragments in the e.i. spectrum, an ion at 151 (168–$NH_3$).

In order to make a decision as to the position of the hydroxy and the methoxy groups, 2 authentic samples (Calbiochem), namely 3-hydroxy-4-methoxyphenethylamine and 4-hydroxy-3-methoxyphenethylamine **2** were compared to **1**. The 1st but not the 2nd compound was found to have physicochemical properties (HRMS, TLC) identical to those of fraction **1**.