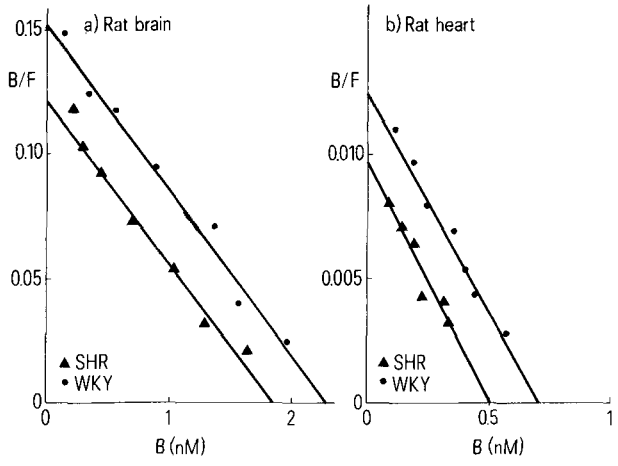
and 10 min for heart. Specific binding is defined as the difference between total binding and nonspecific binding which is measured in the presence of an excess of cold ouabain (0.1 mM). After filtration the radioactivity trapped on the filters (GF/C Whatman) was determined.



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.

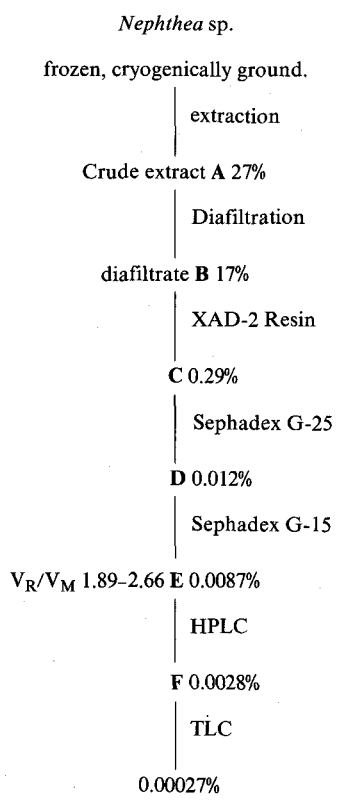
Routine bioassays of aqueous extracts of marine organisms revealed that the aqueous extract **A** of a soft coral *Nephthea* sp[2] (Coelenterata: Octocorallia) increased heart rate and blood pressure when administered i.v. to DOCA-salt hypertensive, pentobarbitone-sodium anaesthetised rats. Histamine, 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**.



Separation scheme for isolation of the cardioactive constituent from *Nephthea* sp.

The tachycardia produced by **1** (1 mg kg^{-1}) was 40 ± 5 beats min^{-1} (mean $\pm$ SEM, 3 rats) whereas **2** (1 mg kg^{-1}) only raised heart rate by 15 ± 1 beats min^{-1}, the difference being highly statistically significant ($p < 0.05$). The rise in blood pressure produced by **2** (1 mg kg^{-1}; 34 ± 2 mm Hg) was not significantly greater than that produced by **1** (1 mg kg^{-1}; 29 ± 6 mm Hg). Administration (i.v. 1 mg kg^{-1}) of **1** and **2** to 3 normotensive rats produced analogous rises in heart rate (**1**, 37 ± 2 beats min^{-1}; **2**, 14 ± 2 beats min^{-1}) and blood pressure (**1**, 12 ± 2 mm Hg; **2**, 12 ± 1 mm Hg).

There are no previous reports of the occurrence of **1** in marine organisms. However, **1** is known to occur in cacti[4] and has been detected in human urine[5]. The cardiovascular properties of **1** are previously unreported but **1** has been implicated in central nervous system disorders such as Parkinsonism[6,7], hyperactivity[8], schizophrenia[5] and dyskinesias[9].

1 Principal author and from whom reprints may be requested.
2 RRIMP Museum specimen FN 1922, collected from North West Reef off Tryon Island, Queensland, Australia. We thank P.A. Alderslade for taxonomy.
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Increased vascular prostacyclin activity in rats after endotoxin administration[1]

S. Villa, G. de Gaetano and N. Semeraro[2]

Laboratory of Cardiovascular Clinical Pharmacology, Istituto di Ricerche Farmacologiche 'Mario Negri', Via Eritrea, 62, I-20157 Milan (Italy), 16 September 1980

Summary. Endotoxin did not interact in vitro with prostacyclin activity but stimulated its release from vascular tissues when administered in single doses to rats 30 min before testing.

Over the last decade several investigators have found increased levels of prostaglandins (PG), particularly of the E and F type, in endotoxemia[3-6]. There is evidence that PGs mediate some in vivo effects of endotoxins such as fever[7], abortion[8] and early phases of shock[9]. More recently, elevated blood levels of respectively thromboxane B_2[10] and 6-keto-$PGF_{1\alpha}$ the stable derivative of PGI_2 (prostacyclin)[11], have been observed after administration of large amounts of endotoxin to rats and rabbits. Whether the high blood levels of 6-keto-$PGF_{1\alpha}$ are the consequence of increased vascular production of PGI_2 - a potent vasodilator and inhibitor of platelet aggregation[12] - has not yet been established.

We report here that vascular tissues from rats given low doses of endotoxin show increased PGI_2 activity. This may result from a complex in vivo interaction between endotoxin and vessel wall since it could not be demonstrated in vitro.

Materials and methods. Male CD-COBS rats (250–300 g b.wt) from Charles River, Calco, Italy, were used. PGI_2 activity was measured in arterial and venous tissues as platelet aggregation inhibitory potency[13]. Saline suspensions of the following endotoxins (lipopolysaccharides (LPS)), were prepared: *Salmonella minnesota* LPS, and *Escherichia coli* 0111: B4 LPS, W. (Difco Laboratories, Detroit, Michigan, USA).

For in vitro experiments, vascular rings from untreated rats[13] were incubated at room temperature for 5 min with different concentrations of endotoxins (100–500 µg/ml) or saline as a control. Afterwards, the supernatant was tested for PGI_2 activity[13]. Synthetic prostacyclin, sodium salt (Upjohn Co., Kalamazoo, USA) was dissolved in ethanol and diluted in 0.05 M Tris buffer pH 9.0 just before use. Mixtures of prostacyclin and endotoxin at different concentrations were incubated for 5 min at room temperature and tested for their antiaggregating activity.

For ex vivo experiments, endotoxin suspensions (0.25–1.0 mg/kg b.wt) were given i.v. and the animals were killed at intervals thereafter. In 1 group of rats 2 endotoxin injections were given 24 h apart and the animals were killed