The First Demonstration of CGRP-Immunoreactive Fibers in Canine Hearts: Coronary Vasodilator, Inotropic and Chronotropic Effects of CGRP in Canine Isolated, Blood-Perfused Heart Preparations

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Abstract—CGRP-immunoreactive nerve fibers were histologically stained in the endocardium and perivascular layer of coronary vessels of canine hearts. To examine the physiological role of the CGRP in the heart function, effects of exogenous CGRP on the hearts were studied using canine isolated, blood-perfused heart preparations. CGRP exerted dose-related potent vasodilator effects with a minimal increase in the developed tension of the papillary muscle, but slightly decreased the sinoatrial rate. The vasodilator effects were unaffected by the pretreatment of either atropine or propranolol. These specific functional effects on the coronary artery are well in accordance with the anatomical localization of CGRP. Taken together, CGRP seems to play an important role in the regulation of coronary vascular tone, while it has only a small functional role in inotropism and chronotropism in canine hearts.

Materials and Methods

Mongrel dogs of either sex, weighing approximately 10 kg, were anesthetized with pentobarbital sodium (30 mg/kg, i.v.), and then they were given heparin calcium (500 U/kg, i.v.). The hearts were excised after exsanguination and plunged into cold Tyrode’s solution kept at 4°C for immunohistochemical and functional studies.

1. Immunohistochemical studies

Small pieces of tissue were dissected from...
the right atrium and the right ventricle, embedded in OCT compound (Miles Laboratories) and then immediately frozen with liquid nitrogen. Sections were cut 4 μm thick by the cryostat, and then attached on glass slides, fixed with cold acetone (4°C) for 10 min, and then washed with cold phosphate-buffered saline (PBS). The specimens were immunostained by the indirect immunofluorescence method. The specimens were treated with anti-rat CGRP rabbit antiserum (1:100, 1:1000; Cambridge Research Biochemicals, Ltd.) for 24 hr at room temperature and then washed with PBS and treated sequentially with fluorescein isothiocyanate-conjugated anti-rabbit IgG (1:10, M.B.L.). The preparations were washed with PBS, mounted in glycerin and observed under an Olympus fluorescence microscope with filters selective for fluorescence. Normal rabbit antiserum was used as a negative control.

2. Functional studies

Blood-perfused heart preparations: Eight papillary muscle preparations and eight sinoatrial node preparations were made from the excised hearts. These preparations were essentially the same as those described by Endoh and Hashimoto (23) and Kubota and Hashimoto (24), respectively.

Briefly, the papillary muscle preparation consisted of the anterior papillary muscle of the right ventricle attached to the interventricular septum. The anterior septal artery, the nutrient artery to the papillary muscle, was directly cannulated. Bipolar electrodes were sutured onto the His bundle region close to the tricuspid valves. The papillary muscle preparation was driven through the electrodes with rectangular pulses of 1–3 V (about 20% above the threshold voltage) and 5 msec duration at frequency of 120 beats/min using an electronic stimulator (Dia Medical System, DHM-226-3) and an isolation unit (Dia Medical System, DPS-110).

The sinoatrial node preparation consisted of the entire right atrium. The sinus node artery was cannulated through the right coronary artery. Bipolar recording electrodes were sutured onto the right atrium close to the sinoatrial node.

Donor dogs: Adult mongrel dogs of either sex, weighing 14–27 kg, were used as donor dogs which were anesthetized initially with sodium pentobarbital (30 mg/kg, i.v.) and supplemented with 50 mg when necessary. At the start of cross circulation, heparin calcium at 500 U/kg, i.v., was given and 200 U/kg, i.v., was supplemented every hour. Respiration was controlled with a dog respirator (Harvard Apparatus, 607), and the systemic blood pressure and heart rate were monitored continuously with a polygraph (NEC San-ei, 361–6).

Cross circulation: Both preparations were placed in double-wall glass jackets maintained at 38°C by circulating warm water and perfused with arterial blood from the carotid artery of the donor dog. Perfusion pressure was kept at 100 mmHg with a peristaltic pump (Cole-Parmer, 7553-00) and a Starling’s pneumatic resistance placed parallel to the perfusion system. Venous blood from the preparations and excess blood passing through the pneumatic resistance were collected in a blood reservoir and were returned to the jugular vein of the donor dog.

Parameters: CBF through the anterior septal artery was measured with an electromagnetic flowmeter (Nihon Kohden, MVF-1100). DT of the papillary muscle, preloaded with a 2-g weight, was measured isometrically using a force displacement transducer (Dia Medical System, DRM-100S). SAR was measured with a cardiotachograph (NEC San-ei 1321) triggered by the atrial electrogram. These three parameters were recorded on a rectilinear recorder (NEC San-ei, 8K231 S).

Drugs and statistics: Drugs used were CGRP (rat, Peninsula Laboratories, Inc.), acetylcholine chloride (Daiichi), atropine sulfate (Tansabe), dl-isoproterenol hydrochloride (Nikkenkagaku), propranolol hydrochloride (ICI Pharma-Sumitomo) and were dissolved in saline. The data were presented as the mean±S.E.M. Student’s t-test for paired data was used, and P values less than 0.05 were considered significant.

Results

1. Immunohistochemical studies

In both the atrium and ventricle, bundles of CGRP-I nerves were found to be running along the coronary vessels, and fine varicose...
Fig. 1. CGRP-immunoreactive (CGRP-I) nerves in the canine hearts. (A) Numerous CGRP-I nerves were observed within the perivascular layer of the coronary vessels (V) of the right atrium. Few CGRP-I nerve fibers were present within the myocardium. (×200). (B) Numerous CGRP-I nerves were observed within the endocardium and perivascular layer of the small coronary vessels (V) of the right ventricle. Few CGRP-I nerve fibers were present within the myocardium. (×100). (C) Control tissue. No fibers with immunoreactivity were found. (×200).

fibers were found in the endocardium (Fig. 1, A and B), while few CGRP-I nerves were found within the myocardium (Fig. 1, A and B). In the control tissues, no fibers with immunoreactivity were observed (Fig. 1C).

2. Functional studies

One hour after the start of the blood-perfusion, the papillary muscle preparation showed a CBF of 3.9±0.6 ml/min (n=7) and a DT of 5.7±0.9 g (n=8), while the sinoatrial preparation showed a spontaneous SAR of 101±6 beats/min (n=7), which persisted unchanged over 8 hours until the end of the experiments. Effects of CGRP were completely reversible 10 to 15 min after a single shot of the maximum dose. Injections of 10 or 30 μl of saline used for solvent did not affect any of the variables measured.

Effects of CGRP on CBF, DT and SAR: CGRP (0.01–10 μg) was injected into each nutrient artery with a microsyringe (Terumo) for a period of 4 sec. Maximal changes in CBF, DT and SAR after drug administration were expressed as percent of their basal values before injection. As seen in Fig. 2, CGRP produced a dose-dependent increase in CBF and DT and decrease in SAR. Figure 3 summarizes the results obtained from 8 papillary muscle and 7 sinoatrial node preparations. The highest dose of CGRP (10 μg) increased the CBF by 100.6±38.3% with a minimal associated increase in DT (6.0±0.2%) and a decrease in SAR (−7.0±1.4%). The ED50 for % change in CBF was 2.0 μg.

Effects of atropine and propranolol on CGRP-induced coronary vasodilator effect: As the blocking effects of a single injection of atropine and propranolol were reported to persist for 20–30 min in these preparations (23, 24), each drug was administered within 10 min in a series of experiments. Effects of atropine (1 μg) on CGRP (3 μg)-induced coronary vasodilator responses are shown in Fig. 4. The responses to CGRP were unchanged after cholinergic blockade with atropine at a dose sufficient to attenuate significantly the coronary vasodilator effect of ACh (1 μg). Effects of propranolol (10 μg) on CGRP (3 μg)-induced coronary vasodilator response are shown in Fig. 5. The responses to CGRP were also unchanged after beta-adrenergic blockade with propranolol.
Fig. 2. Original tracings showing the effects of CGRP (0.01–10 \mu g). (A) CGRP caused a significant increase in blood flow through the anterior septal artery accompanied with a slight increase in developed tension of the papillary muscle. (B) CGRP caused a slight decrease in sinoatrial rate.

Fig. 3. Dose-response curves for % changes in coronary blood flow, developed tension and sinoatrial rate.
Cardiac Effects of Circulating CGRP

Discussion

In the present experiments, immunohistochemical studies showed that CGRP-I nerves existed and localized within the endocardium and the perivascular layer in canine hearts. Also, the functional studies using the isolated blood perfused heart preparations demonstrated that circulating CGRP had a potent dose-dependent vasodilator effect with few, if any, inotropic and chronotropic effects.

Increases in the exogeneous dose of CGRP by single injections of synthetic CGRP into each nutrient artery caused a potent, dose-dependent coronary vasodilation which was not antagonized by either atropine or propranolol treatment. The vasodilator effect of CGRP is therefore considered not to be through sympathetic and parasympathetic mechanisms and supports the possibility that CGRP may play a role in non-adrenergic and non-cholinergic regulation of the coronary artery tone (4, 6).

Injections of CGRP into the anterior septal artery had almost no effect on the DT of papillary muscle, which is apparently in good agreement with the results observed in rat, guinea pig and rabbit papillary muscle (3, 7, 14, 18). However, positive inotropic effects of CGRP on rat, guinea pig and human atria (4–6, 14, 16, 17) and on porcine ventricular muscle (26) have been reported. On the other hand, injection of CGRP into the right coronary artery had almost no effect on the SAR of the sinoatrial node preparation, which is in good agreement with the recent in vivo observation by Rigel (27). This observation is in a sharp contrast with the potent positive chronotropic effects in rat and guinea pig atrium (4–6, 14, 17). These varied observations may be due to the species difference in the CGRP receptor distribution within the heart (9), and there may be little functional CGRP receptor in the myocardium of the canine hearts, although precise CGRP receptor distributions in the canine heart are not known. Instead, the absence of a cardiac effect of CGRP may suggest a more likely role of other neuropeptide in canine hearts. In particular, recent observations by Rigel in-
dicate that vasoactive intestinal polypeptide and/or peptide histidine isoleucine, but not CGRP, mediate non-adrenergic and non-cholinergic tachycardia in the dog (27).

Exogeneously applied CGRP has a potent dilator activity in the coronary artery, but it has almost no effects on DT and SAR. These functional results are well in accordance with the anatomical localization of CGRP. In conclusion, our comparative analysis of histological and functional studies indicates that in the canine heart, CGRP plays a role in causing coronary vasodilation with little cardiotonic effect. For further studies to examine the regulation by CGRP of the coronary circulation, canine hearts should provide a suitable model system.

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