CNP causes receptor-mediated positive dromotropic effects in anesthetized dog hearts

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C-TYPE NATRIURETIC PEPTIDE (CNP) is a newly identified 22-amino acid peptide that demonstrates structural similarity to the cardiac hormones atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) (7). This peptide is widely distributed within the vascular endothelium (3) and may play a role in the regulation of vascular tone (2). Recently, CNP mRNA has been detected in the rat heart (12), and the existence of CNP has been demonstrated in the human ventricle (13).

Both ANP and BNP function biologically via a natriuretic peptide type A receptor (NPR-A) that is highly expressed in the kidney (11). The NPR-B is preferentially expressed in cardiac tissue and vascular smooth muscle, whereas the NPR-A is highly expressed in the kidney (11).

Recently, Beaulieu et al. (1) observed that CNP but not ANP increased sinus rate in anesthetized and isolated dog hearts. However, there is no available report of the effects of CNP on atrioventricular (AV) conduction. Therefore, we investigated the dromotropic effects of CNP injected directly into the AV node artery in autonomically decentralized hearts in open-chest, anesthetized dogs. We observed a positive dromotropic response to CNP. Thus, to determine whether the positive dromotropic response to CNP is mediated by guanylyl cyclase-linked natriuretic receptors, we examined the effects of HS-142–1 on positive cardiac responses to CNP in anesthetized dogs. HS-142–1 is an inhibitor of the guanylyl cyclase-linked natriuretic peptide receptors, i.e., NPR-A and NPR-B (9).

METHODS

The animal experiments were approved by the Shinshu University School of Medicine Animal Studies Committee.

Preparations. Six mongrel dogs of either sex, weighing 15–20 kg, were anesthetized with pentobarbital sodium (35 mg/kg iv). A tracheal cannula was inserted, and intermittent positive-pressure ventilation was started by a respirator (model 607, Harvard Apparatus, Millis, MA) with room air. The chest was opened transversely at the fifth intercostal space. Cervical vagus nerves were isolated bilaterally via a midline neck incision and crashed with tight ligature. Each stellate ganglion was also isolated and ligated tightly at its junction with the ansa subclavia. These maneuvers remove almost all tonic neural activity to the heart (8).

Two bipolar electrodes, each used to record the atrial electrogram and to pace the atrium, were placed on the epicardial surface of the right atrial appendage, and a bipolar electrode was placed on the base of the epicardial surface of the right ventricle to record ventricular electrogram. Atrial pacing at a fixed atrial interval (400 ms) was performed by an electrical stimulator (SEN 7103, Nihon-Kohden, Tokyo, Japan), which delivered a 1-ms rectangular pulse at twice the fixed diastolic voltage threshold. AV conduction time (AV interval) was measured with an AV interval counter (ET-601G, Nihon-Kohden) that detected the upstroke of the atrial and ventricular electrograms. The systemic arterial blood pressure was recorded from the left femoral artery by a pressure transducer. AV interval and systemic arterial blood pressure were recorded and displayed on an oscillograph (model RTA-1200, Nihon-Kohden). The left femoral vein was cannulated for

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drug injection and for physiological saline infusion to adjust spontaneous fluid losses.

Direct perfusion of the AV node artery was prepared as previously described (4). Polyethylene tubing (outer diameter 2.2 mm) was tapered to fit a cannula, the tip of which had an outer diameter of 0.5–1 mm. Rubber tubing was connected to the shank of the cannula for injection of drug solution. The distal branch of the left circumflex coronary artery was carefully isolated from the origin of the AV node artery and cannulated with the cannula. The AV node artery was then perfused with heparinized blood from the right femoral artery. The perfusion pressure could be maintained constant at 90 mmHg by means of shunting the excess blood to the blood reservoir through a pneumatic resistance that was placed in parallel with the perfusion system. The perfusion blood flow rate was measured in the extracorporeal circuit of the perfusion system by an electromagnetic flowmeter (MFV-2100, Nihon-Kohden) and displayed on an oscillograph. Heparin sodium (500 U/kg iv) was administered at the beginning of the perfusion and was given subsequently (200 U/kg) at 1-h intervals.

Experimental protocols. We carried out two series of experiments after 30-min stabilization from the surgical procedures. In the first series, to examine the effects of CNP on AV conduction, we studied the changes in AV interval and coronary blood flow rate in response to CNP (0.1–3 nmol, n = 6 dogs) injected into the AV node artery of autonomically decentralized hearts in open-chest, anesthetized dogs. Additionally, isosorbide dinitrate (ISDN, 64 nmol; n = 5 dogs) was injected directly into the AV node artery to evaluate the effect of changes in coronary blood flow on AV conduction.

In the second series, to determine whether the positive dromotropic response to CNP is mediated by guanylyl cyclase-linked natriuretic peptide receptors or β-adrenoceptors, we examined the effects of HS-142–1 (2 mg, n = 6 dogs), a guanylyl cyclase-linked natriuretic peptide receptor antagonist, or propranolol (1 mg/kg iv, n = 3 dogs) on the positive dromotropic response to CNP (3 nmol) 30 min after determination of control responses to CNP. The responses to CNP were observed 2 min after each blocker treatment.

Enough recovery time (usually 30 min) after injection of CNP was allowed to avoid the effects of the former injection of CNP on the effects of the following injection of CNP, because our preliminary experiments showed that CNP caused tachyphylaxis.

Drugs. Drugs were mixed fresh for each experiment. Human CNP-22 (Peptide Institute, Osaka, J apan) was dissolved in distilled water, kept frozen at −20°C as stock solution, and diluted immediately before use. A nonpeptide natriuretic peptide receptor antagonist, or propranolol (1 mg/kg iv, n = 3 dogs) on the positive dromotropic response to CNP (3 nmol) 30 min after determination of control responses to CNP. The responses to CNP were observed 2 min after each blocker treatment.

Statistical analysis. All data are shown as maximum change in response to each drug and are expressed as means ± SE. An analysis of variance with Bonferroni’s test was used for statistical analysis of multiple comparisons of data. Student’s t-test for unpaired data was used for comparison between the two groups. P values of <0.05 were considered statistically significant.

RESULTS

When CNP (3 nmol) was injected into the AV node artery in an autonomically decentralized heart in an open-chest, anesthetized dog, CNP decreased the AV interval (Fig. 1, left). Table 1 shows summarized data of the effects of CNP on the AV interval and coronary blood flow rate in six dogs. CNP (0.1–3 nmol) decreased the AV interval (P < 0.001) and increased the coronary artery blood flow rate (P < 0.001) dose dependently. On the other hand, ISDN at a dose of 64 nmol injected into the AV node artery did not affect the AV interval significantly but increased the coronary artery blood flow rate by 2.5 ± 0.8 ml/min in five experiments.

To investigate whether the positive dromotropic response to CNP was mediated by guanylyl cyclase-linked natriuretic peptide receptors, we studied the effects of HS-142–1 (2 mg) on the positive dromotropic response to CNP (3 nmol) in six autonomically decentralized hearts in open-chest, anesthetized dogs. After treatment with HS-142–1, the positive dromotropic response to CNP was attenuated in an anesthetized, open-chest dog (Fig. 1, right). Figure 2 shows summarized data of the effects of HS-142–1 on the positive dromotropic response to CNP in six dogs. HS-142–1 inhibited the positive dromotropic response to CNP significantly (P < 0.001). HS-142–1 also attenuated the increases in coronary blood flow rate in response to CNP (P < 0.01) in the same six dogs (Fig. 2). HS-142–1 did not affect the basal AV interval during the experiment.

When we studied the effects of propranolol (1 mg/kg iv) on the positive dromotropic response to CNP (3 nmol) and norepinephrine (0.3 or 1.0 nmol) in three...
anesthetized dogs, propranolol did not affect the positive dromotropic response to CNP but abolished the decreases (−35 ± 9.5 ms) in AV interval in response to norepinephrine.

When each drug was injected into the AV node artery, systemic arterial blood pressure did not change significantly.

DISCUSSION

We demonstrated in the present study that CNP injected directly into the AV node artery decreased AV interval in autonomically decentralized hearts in open-chest, anesthetized dogs (Fig. 1 and Table 1). HS-142–1 blocked the positive dromotropic response to CNP (Fig. 2). However, the positive dromotropic response to CNP was not inhibited by propranolol in doses that completely inhibited the norepinephrine-induced positive dromotropic response. HS-142–1 is reported to be a specific natriuretic peptide receptor antagonist (9). The affinity cross-linking study demonstrated that HS-142–1 specifically abolished labeling of the 135-kDa band that was derived from labeling of the guanylyl cyclase-linked natriuretic peptide receptors. However, HS-142–1 had no effect on labeling of the 60-kDa band that was derived from the guanylyl cyclase-free receptors, i.e., NPR-C. HS-142–1 also inhibited cGMP production stimulated by ANP, BNP, and CNP with almost equal potency in PC12 cells. From the present results, therefore, we suggest that CNP decreases the AV interval mediated by the guanylyl cyclase-linked natriuretic peptide receptors but not by the guanylyl cyclase-free receptors or β-adrenergic mechanism in the dog heart. Additionally, CNP injected into the AV node artery increased coronary blood flow rate in anesthetized dog hearts (Table 1). HS-142–1 attenuated the increase in flow rate in response to CNP (Fig. 2), indicating that coronary vasodilation in response to CNP is mediated by guanylyl cyclase-linked natriuretic peptide receptors in the dog heart. This observation is consistent with the previous study that reported that CNP worked as a coronary vasodilator through activation of cGMP by way of particulate guanylyl cyclase in isolated canine coronary artery or anesthetized dog heart preparations (14).

Recently, Koller et al. (6) described ligand specificity of the two different subtypes of guanylyl cyclase-linked natriuretic peptide receptors, i.e., NPR-A and NPR-B. NPR-A has high affinity for ANP and BNP, whereas NPR-B binds only CNP with high affinity. Dose-response curves for stimulation of guanylyl cyclase of NPR-A and NPR-B demonstrated that both ANP and BNP could effectively stimulate NPR-A, and that BNP was 10-fold less potent than ANP (5). In contrast, CNP did not significantly increase intracellular cGMP in cells expressing NPR-A. In NPR-B-expressing cells, only CNP could effectively stimulate cGMP production (5). Therefore, it is likely that the positive dromotropic effects of CNP are mediated by NPR-B in the dog heart.

Our present results showed that CNP decreased AV interval with increases in coronary artery blood flow rate (Table 1). When HS-142–1 attenuated the positive dromotropic response to CNP, it also attenuated the increases in flow rate in response to CNP (Fig. 2). To study the possibility that increases in coronary flow rate evoked by CNP affect the decrease in AV interval, we investigated the effects of ISDN injected into the AV node artery. ISDN and ANP induced increases in tissue cGMP as well as coronary vasodilation in isolated canine coronary artery (10). In our present study, ISDN did not alter AV interval, despite the fact that the increase in coronary blood flow rate induced by ISDN at 64 nmol was similar to that of CNP at 3 nmol. Therefore, we suggest that the positive dromotropic response to CNP is not mediated by the increase in coronary blood flow, e.g., a local reflex mechanism caused by coronary vasodilation, in the dog heart but that it results from the direct effect of CNP on the conduction system.

When HS-142–1, a natriuretic peptide receptor blocker, was injected into the AV node artery of anesthetized dog hearts, it did not affect the basal AV interval. Although CNP circulates in low picomolar concentrations in canine plasma (2), circulating CNP may not have a potential role in AV conduction in physiological states. However, in the present study we demonstrated that CNP injected into the AV node artery affected AV interval. CNP mRNA has been detected in the rat heart (12), and the existence of CNP has been demonstrated in the human ventricle (13). Therefore, CNP may have effects on AV conduction as a paracrine hormone.

In conclusion, we have demonstrated for the first time that CNP decreased AV interval in autonomically decentralized hearts in open-chest, anesthetized dogs. HS-142–1, a guanylyl cyclase-linked natriuretic peptide receptor antagonist, but not propranolol, blocked the decrease in AV interval in response to CNP, indicating that the positive dromotropic response to CNP was mediated by a guanylyl cyclase-linked natriuretic peptide receptor, NPR-B.

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