Atrial natriuretic peptide and mechanisms of cardiovascular control. Role of serotonergic receptors

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Deliva, Robin Donna, and Uwe Ackermann. Atrial natriuretic peptide and mechanisms of cardiovascular control. Role of serotonergic receptors. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R711–R717, 1998.—Atrial natriuretic peptide (ANP) inhibits renal sympathetic nerve activity (RSNA), provided the vagi are intact. Afferents from chemosensitive cardiopulmonary receptors are specifically required. Such receptors produce the Bezold-Jarisch reflex, are prominent on the ventricular epicardium, and are richly supplied with 5-hydroxytryptamine type 3 (5-HT3) receptors. We tested the hypothesis that epicardial and are richly supplied with 5-hydroxytryptamine type 3 (5-HT3) receptors. Through a special catheter, anesthetized, sinoaortically denervated rats received pericardial test injections of ANP (28-amino acid rat ANP; 100 and 1,000 ng) in the presence or absence of 5-HT3 antagonist (Ondansetron, 20 µg/kg; amino acid rat ANP; 100 and 1,000 ng) in the presence or absence of 5-HT3 antagonist (Ondansetron, 20 µg/kg; n = 9).

In other groups we observed the effects of systemic ANP while blocking either epicardial or systemic 5-HT3 receptors. Arterial blood pressure (ABP), heart rate, and RSNA were recorded continuously. Intravenous ANP (100 or 200 ng) decreased ABP and RSNA significantly. In contrast, intrapericardial ANP (100 or 1,000 ng) caused no significant fall in ABP or RSNA. Both intravenous and pericardial Ondansetron reduced the effects of intravenous ANP significantly, but the intravenous antagonism was significantly greater. We conclude that epicardial chemosensitive afferents are not sensitive to ANP and that sympathoinhibitory effects of ANP arise from a 5-HT3 agonist that cannot be produced when ANP is confined to the pericardial space.

THE CARDIOVASCULAR EFFECTS OF ATRIAL NATRIURETIC PEPTIDE (ANP) INCLUDE ACTIONS ON THE AUTONOMIC NERVOUS SYSTEM, THE HEART, AND PERIPHERAL BLOOD VESSELS. THE NET OUTCOME OF THESE ACTIONS IS SUPPRESSION OF REGIONAL SYMPATHETIC OUTFLOW (9, 10, 21, 25), LACK OF APPROPRIATE REFLEX TACHYCARDIA DURING EPISODES OF HYPOTENSION (2), AND, AS NOTED IN HUMAN STUDIES, OCCASIONAL PROFOUND BRADYCARDIA WITH FEELINGS OF FAINTNESS (4). THESE OBSERVATIONS IMPLY A SIGNIFICANT INTERACTION BETWEEN ANP AND CARDIOVASCULAR REGULATORY MECHANISMS, AN IMPLICATION THAT IS FURTHER STRENGTHENED BY THE FINDING THAT ANP RELAXES α-ADRENERGICALLY CONSTRICTED RESISTANCE VESSELS (8) BUT IS WITHOUT EFFECT ON THEIR MYOGENIC RESPONSE (8, 17).

ANP HAS BEEN SHOWN TO ACT AT SEVERAL NEUROENDOCRINE TARGET SITES. AMONG THEM ARE CARDIAC PARASYMPATHETIC NERVE TERMINALS (2, 3) AND SYMPATHETIC NERVES OF THE SKELETAL MUSCLE VASCULAR BED (9). OUR EARLY STUDIES OF THE MECHANISMS BY WHICH ATRIAL NATRIURETIC FACTORS INFLUENCE CARDIOVASCULAR FUNCTIONS IN WHOLE ANIMALS DEMONSTRATED THAT SUCH INFLUENCES WERE GREATLY DIMINISHED WHEN THE VAGI WERE CUT (1). OUR RESULTS LED US TO SPECULATE THAT ATRIAL FACTORS TRIGGER AUTONOMIC RESPONSES BY INTERACTING WITH CHEMOSENSITIVE CARDIOPULMONARY NEURONS. THIS SPECULATION HAS BEEN SUPPORTED BY OTHERS WHO FOUND THAT VAGOTOMY ABOLISHES THE SUPPRESSION OF RENAL SYMPATHETIC NERVE ACTIVITY (RSNA) AFTER SYSTEMIC INJECTION OF ANP (10, 25) AND DEMONSTRATED THAT THESE EFFECTS OF ANP ON CARDIORENAL REGULATION ARE DUE SPECIFICALLY TO VAGAL C FIBERafferents (21). THESE NONMYELINATED FIBERS ARISE FROM MECHANOSENSITIVE OR CHEMOSENSITIVE NEURONS (5, 7), BUT IT IS THE CHEMOSENSITIVE FIBERS THAT ARE INVOLVED IN THE SYMPATHOINHIBITORY EFFECTS OF ANP (21). VAGAL C FIBERS ARISE EXTENSIVELY FROM THE VENTRICULAR EPICARDIAL SURFACE (24), BUT ARE FOUND ELSEWHERE (6). THEY ARE INVOLVED SIGNIFICANTLY IN BOTH NORMAL PHYSIOLOGICAL CONTROL OF CARDIOVASCULAR FUNCTION (14) AND IN PATHOPHYSIOLOGICAL MECHANISMS, INCLUDING THE CIRCULATORY EVENTS THAT ACCOMPANY CORONARY ISCHEMIA AND REPERFUSION (27). THE PROMINENT EFFECT OF VAGAL C FIBER ACTIVATION IS THE BEZOLD-JARISCH REFLEX. IT IS CHARACTERIZED BY BRADYCARDIA, HYPOTENSION, AND SPECIFIC SUPPRESSION OF RSNA (18, 26). THE LATTER HAS BEEN USED BY SEVERAL INVESTIGATORS AS AN INDEX OF CIRCULATORY CONTROL BY CARDIAC AFFERENTS IN RATS (18). VAGAL CHEMOSENSITIVE C FIBERS ARE RICHLY SUPPLIED WITH 5-HYDROXYTRYPTAMINE TYPE 3 (5-HT3) RECEPTORS (29). THEY ARE, THEREFORE, ACTIVATED BY SEROTONIN. THEY ARE ALSO ACTIVATED BY A VARIETY OF OTHER AGENTS, INCLUDING BRADYKININ (12), PROSTAGLANDINS (27), OXYGEN-DERIVED FREE RADICALS (27), NICOTINE (23), AND CAPSAICIN (6). THE 5-HT3-SELECTIVE AGONIST PHENYL BIGUANIDE (PBG) (5) IS A SPECIFIC ACTIVATOR OF C FIBER CHEMOSENSORS AND IS WITHOUT EFFECT ON C FIBER MECHANORECEPTORS (5).

The studies described here had the primary objective of determining the role of 5-HT3 serotonergic receptors in the sympathoinhibitory reflexes elicited by ANP and the secondary objective of determining whether ventricular chemosensitive fibers are specifically involved.

All previous studies involving possible interactions between ANP and vagal chemosensitive afferents have used intravenous injections of the peptide. It is not clear whether systemic elevations of ANP lead to increased levels in the pericardial space, the region showing the highest concentration of serotonergic, vagal C fiber afferents (24). The question of whether or not direct application of ANP to vagal C fibers can elicit a Bezold-Jarisch reflex, characterized by hypotension, bradycardia, and decreased RSNA, has not been previously addressed.

MATERIALS AND METHODS

Surgical Preparation

Experiments were performed on 66 male Sprague-Dawley rats (Charles River) weighing 357 ± 8 g (mean ± SE). They
were initially anesthetized with pentobarbital sodium (50 mg/kg ip) and underwent a tracheotomy with PE-390 tubing heated and pulled to an appropriate diameter. The right femoral vein was cannulated with PE-90 tubing for injection of drugs. The right femoral artery was cannulated with PE-50 tubing for measurement of arterial blood pressure (ABP) (Sensormedics, type 4-327 H transducer) and derivation of heart rate (HR). A PE-50 catheter was placed into the right femoral vein so that methohexital sodium could be constantly infused at a rate of 0.25 mg/min to maintain anesthesia as the effects of pentobarbital sodium diminished with time. Experiments in groups 9 and 10 (see Protocol) were conducted using 1-nactin (5-sec-butyl-5-ethyl-2-thiobarbituric acid; 100 mg/kg ip) before we were told (R. Veelken, personal communication) of the stronger nerve signals obtainable with methohexital sodium.

To avoid confusing baroreflex effects, all rats underwent the sinoaortic denervation procedure of Krieger and Marselli (13). Briefly, the neck was opened to expose the carotid bifurcation. The carotid sinus nerves and glossopharyngeal and superior laryngeal nerves were isolated and tied bilaterally. The recurrent laryngeal nerves, running on either side of the trachea, were also tied. In rats with an identifiably separate aortic depressor nerve (ADN), running along the carotid arteries and the vagus as a sympathetic trunk, the ADN was tied near its junction with the superior laryngeal nerves. Sinoaortic denervations were considered successful when the HR did not increase by more than 15–20 beats/min in response to fall in mean ABP (30–50 mmHg) induced by a bolus intravenous injection of nitroprusside. When drugs were applied to the epicardial ventricular surface they were delivered via an intrapericardial catheter put in place while the chest was closed. This infusion/withdrawal catheter was prepared by tying a knot at the midpoint of a 3-cm length of Silastic tubing (0.012 in. ID; 0.025 in. OD). The knot divided the tubing into two distinct, joined catheters, one for infusion and the other for withdrawal of fluid. The tube was perforated close to the knot five times on the withdrawal side and twice on the infusion side. The free end of each line was fitted snugly over the end of a 20-cm length of PE-10 polyethylene tubing, leaving less than 13 µl of dead space in each arm of the tubing. A 20-cm length of 14-g stainless steel tubing acted as a stylet-like introducer. The Silastic knot was placed over one end of the stainless steel tubing, and each catheter arm was held snugly parallel to the steel tubing, inserted at the level of the submaxillary glands just lateral to the trachea, and advanced forward, underneath the sternomastoid muscle, toward and then past the thymus gland to the level of the heart. The impacts of the beating heart could be felt as they were transmitted along the stainless steel tubing and indicated correct catheter placement. The stylet was advanced an additional 1 cm after the first pulsations were felt, thereby ensuring that the inflow and outflow arms of the catheter were placed securely inside the pericardial sheath. The metal tubing was then gently rotated back and forth as it was withdrawn, leaving the catheter in place. The catheter was taped to the surgical table to prevent it from being displaced during the experiment.

The left renal artery with the vago-sympathetic trunk was exposed. The left kidney was placed in a kidney cup to reduce mechanical disturbances and a branch of the renal nerve was located near the origin of the renal artery. A small pool of paraffin oil was placed around the nerve. Connective tissue surrounding the nerve was carefully dissected away with a glass rod and the nerve was placed on a thin, bipolar platinum electrode. Nerve signals were amplified 1,000 times with an AC preamplifier (model P15, Grass Instruments), set to a bandwidth of 30 Hz to 10 kHz. The signal was monitored visually by an oscilloscope and was fed through a moving time average circuit consisting of a full-wave rectifier and a low-pass filter whose time constant was set to 100 ms. The output from the low-pass filter as well as the amplified signal from the blood pressure transducer was fed into a data acquisition system (BIOPAC Systems, Goleta, CA) for on-line recording and later analysis. At the end of each experiment, baseline activity was determined after crushing the central end of the nerve so that both afferent activity and noise would be included in the factor that was later subtracted as background activity. In a few experiments we confirmed that the activity recorded from these nerves was, indeed, sympathetic efferent activity. This was done by recording whole nerve activity after intravenous injection of 100 µg/kg body weight of the ganglionic blocking agent pentolinium tartrate and an intravenous test injection of PBG. The observations that ganglionic blockade eliminated all but background activity in the electrodes and that PBG caused no change in the activity indicated that the variable identified as RSNA was not significantly contaminated by afferent or other signals.

**Protocol**

After surgical preparation, each animal was allowed to stabilize for 1 h. After this time a validation injection of 100 µg PBG, a 5-HT<sub>3</sub> receptor agonist, was given in 0.1 ml saline, followed by a 0.1-ml saline flush. This dose was chosen on the basis of Veelken's work (29), and the same amount was injected either intravenously (series A) or pericardially (series B). We had determined in pilot experiments that the introduction of 0.2 ml fluid into the pericardial space had no effect on ABP, HR, or RSNA. The purpose of the PBG validation injections was to confirm in each animal that functioning 5-HT<sub>3</sub> receptors were within reach of the respective injection catheter. The experimental protocol began after a further recovery time of 20–30 min, but only if the PBG injection caused a transient (less than 5 min) fall in mean ABP, HR, and RSNA. There were 10 groups of 6–9 rats each. The rationale for the nature and sequence of injections was to observe the cardiovascular effects of intravenous or intrapericardial ANP in the presence or absence of 5-HT<sub>3</sub> receptors either in the systemic vasculature or the pericardial space. Groups differed from one another by the background or test injections that were given. In each case, succeeding test injections were spaced 20 min apart from the preceding injection. When ANP was given (groups 3, 4, and 7-10), then it was always the last pair of injections and the doses were spaced 20 min apart.

The test injections were as follows.

**Series A: Systemic injections of ANP.** Series A1: Systemically injected ondansetron. Group 1 (n = 6) was the control for group 2. It had a background of intravenous saline. An intravenous test injection of saline (0.1 ml) was followed by intravenous PBG (100 µg). Group 2 (n = 6) was designed for validation of Ondansetron effectiveness as a systemic 5-HT<sub>3</sub> antagonist. It had a background of intravenous Ondansetron (20 µg/kg body wt), but was otherwise identical to group 1. Group 3 (n = 6) compared systemic PBG and ANP effects. It had a background of intravenous saline. Intravenous test injections of ANP were administered at 100 and 200 ng. Group 4 (n = 6) was for determination of systemic ANP effects during systemic 5-HT<sub>3</sub> antagonism. It had a background of intravenous Ondansetron (20 µg/kg body wt), but was otherwise identical to group 3.

**Series A2: Pericardially injected ondansetron.** Group 5 (n = 6) was the control for group 6. It had a background of epicardial saline (0.1 ml) followed by intravenous saline bolus (0.1 ml). Group 6 (n = 6) was designed for validation of Ondansetron effectiveness as an epicardial 5-HT<sub>3</sub> antagonist.
It had a background of epicardial Ondansetron (20 µg/kg body wt), but was otherwise identical to group 5. Group 7 (n = 6) was the control for group 8. It had a background of epicardial saline. Intravenous ANP was infused at 100 and 200 ng. Group 8 (n = 6) was for determination of systemic ANP effects during epicardial 5-HT3 antagonism. It had a background of epicardial Ondansetron (20 µg/kg body wt), but was otherwise identical to group 7.

Series B: Pericardial injections of ANP. Group 9 (n = 9) compared epicardial ANP and PBG effects. It was the control for group 10. It had a background of epicardial saline. Epicardial injections of saline and 100 and 1,000 ng ANP were administered. In a few experiments, 10,000 ng ANP was also injected to rule out insufficient dosing as a cause for observations made at lower doses. Group 10 (n = 9) was designed for determination of epicardial ANP effects during epicardial 5-HT3 antagonism. It had a background of epicardial Ondansetron (20 µg/kg body wt), but was otherwise identical to group 9.

At the end of the protocol time, another test dose of PBG (100 µg) was administered epicardially to ensure that no deterioration of the reflex or change in response of 5-HT3 receptors had occurred. In experiments involving pericardial ANP injections and in view of the failure of such injections to cause changes in the measured variables, we also gave an intravenous injection of 500 ng ANP. Efficacy of the peptide was indicated by a transient fall in ABP.

At the completion of each experiment, the integrity of the pericardial sac was examined by injection of methylene blue dye into the sac (200 µl of a 1 mg/ml solution). After injection, the chest was opened carefully by a side incision. Any rats that revealed leakage of dye from the pericardial space were omitted from subsequent data analysis.

Drugs

PBG was obtained from Aldrich Chemical (catalog no. 16,421–6; Milwaukee, WI), Ondansetron was purchased from Glaxo Canada (no. DIN 0191 1821; Toronto, Canada) as an injectable Ondansetron hydrochloride, and ANP (rat ANP, 28 amino acids) was obtained from Peninsula Laboratories (catalog no. 9103; Belmont, CA). All intrapericardial drugs were administered in a volume of 0.1 ml.

Data Analysis

ABP, HR, and RSNA were recorded continuously by a BioPac data acquisition system (BIOPAC Systems). Control values for each animal were taken as the average that prevailed during the 60-s control interval before the first validation dose of PBG. When comparisons of these values were made among groups, we used the Kruskal-Wallis analysis of variance (ANOVA) on ranks. Subsequent analyses were performed on data that were averaged over an interval chosen so as to include the peak change from the immediate predrug value. To that end, postinjection data for saline or ANP were taken as the average over intervals of 60 s, beginning 60 s after completion of the respective test injection. In view of the
more rapid responses after injection of PBG, responses to this 5-HT3 agonist were averaged over the first 30 s after completion of injection. For each animal the change in RSNA (D RSNA) after drug injections was expressed as a percent change from the predrug value. Absolute values were not compared because of great variability arising, we believe, from variations in nerve size and electrode contact. Statistical analyses of differences in D RSNA were performed on logarithmic transformations of the D RSNA data. Comparisons involving only two groups were made by the Mann-Whitney rank-sum test. Comparisons of ANP effects in the presence or absence of 5-HT3 antagonist were made by Kruskal-Wallis ANOVA on ranks, followed post hoc by Dunn’s test of multiple comparisons when warranted by a significant F statistic. Dunn’s test was chosen over Tukey’s or Student-Newman-Keuls because of unequal sample sizes in several cases. Statistical significance was defined as P < 0.05.

RESULTS

The starting mean ABPs and HRs for the different groups (Table 1) were not significantly different and remained stable throughout the 2-h protocol except for transient changes after injection of test substances like

the 5-HT3 agonist PBG. Figure 1 shows a typical response to a test injection of PBG into the pericardial space. There was a characteristic rapid and short-lived Bezold-Jarisch reflex response, namely, hypotension, bradycardia, and decreased RSNA. Similar observations were made after intravenous PBG, and Fig. 2 shows the changes, averaged over the first 30 s after injection. Both intravenously and pericardially injected 5-HT3 agonist (PBG) depressed mean ABP (D ABP, Fig. 2), HR (D HR, Fig. 2), and RSNA (D RSNA, Fig. 2).

Intravenous or pericardial PBG (each at 100 µg) caused similar decreases in ABP and HR, but intravenous PBG led to a significantly larger inhibition of RSNA than did the same amount of pericardial PBG (Fig. 2). Figure 2 also shows that the depressor actions of intravenous or pericardial PBG were blocked by the 5-HT3 antagonist Ondansetron (groups 2 and 6).

Intravenously injected ANP had no significant effect on HR (Fig. 3). However, compared with an intravenous test injection of saline (groups 1, 2, 5, and 6), ANP (groups 3, 4, 7, and 8) significantly decreased ABP (Fig. 3).

![Fig. 2](http://ajpregu.physiology.org/) Averaged D ABP, D HR, and D RSNA during the first 30 s after intravenous or pericardial bolus injections of the 5-HT3 agonist PBG. In each case, the reference was the mean value prevailing during the 60-s interval before injection. Injections were made into control animals (background = intravenous saline or pericardial saline) or animals that had received a background injection of the 5-HT3 antagonist Ondansetron (background = intravenous Ondansetron or pericardial Ondansetron). Numbers at top identify the relevant group of animals, as described in Protocol. *P < 0.05 vs. respective saline control; †P < 0.05 vs. intravenous PBG test injection.

![Fig. 3](http://ajpregu.physiology.org/) Averaged D ABP, D HR, and D RSNA during the first 60 s after completion of an intravenous bolus injection of saline (IV Saline) or atrial natriuretic peptide (ANP; 100 or 200 ng). In each case, the reference was the mean value prevailing during the 60-s interval before injection. Injections were made 1) into animals that had received a background injection of the 5-HT3 antagonist Ondansetron either intravenously (background = intravenous Ondansetron) or into the pericardial space (background = pericardial Ondansetron) or 2) into animals that had received a control background injection of saline. Either intravenously (background = intravenous saline) or into the pericardial space (background = pericardial saline). The numbers near each vertical bar identify the relevant group of animals, as described in Protocol. *P < 0.05 vs. group identified by bracket; †P < 0.001 vs. test injection of intravenous saline.
3) and RSNA (Fig. 3). These depressor effects were inhibited (Fig. 3; group 4 vs. 3 or 8 vs. 7), but not abolished (Fig. 3; group 4 vs. 2 or 8 vs. 6) by prior injection of Ondansetron. Intravenously injected Ondansetron produced significantly greater inhibition of the depressor effects of either dose of ANP than did pericardial Ondansetron (Fig. 3; group 8 vs. 4). Figure 4 shows time-averaged responses to epicardial administration of saline, PBG, or ANP. The PBG injections were given to animals receiving a background pericardial injection of either saline or the specific 5-HT₃ antagonist Ondansetron. The PBG and Ondansetron data confirm the presence of 5-HT₃ receptors within the pericardial space of these animals. Intrapericardial injections of ANP at 100 or 1,000 ng had no significant effect on ABP, HR, or RSNA either with a saline background (Fig. 4) or with an Ondansetron background (data not shown). Injections of 10,000 ng ANP were also given in some animals. They, too, were without effect on the measured parameters (data not shown).

To confirm the stability of our preparation, a PBG test injection was also given at the end of the experiments, and the subsequent changes in cardiovascular variables were compared with those obtained at the outset of the experiment. In group 1 animals (saline background), the final PBG injection yielded peak ΔABP = −17 ± 4 (SE) mmHg and peak ΔHR = −5 ± 3 beats/min. The magnitudes and temporal patterns of these changes were not different from those observed after the initial PBG injection [peak ΔABP = −17 ± 3 (SE) mmHg and peak ΔHR = −12 ± 7 beats/min]. The change in RSNA after PBG injection at the end of the protocol time was −15.9 ± 3.6%, also not different from the change shown in Fig. 2 (peak ΔRSNA = −24.2 ± 6.4%).

**DISCUSSION**

The effects of ANP on the cardiovascular system include direct receptor-mediated actions on some peripheral blood vessels (20) as well as effects mediated via ANP actions on the autonomic nervous system (9). Thoren et al. (25) and Schultz et al. (21) have provided strong evidence for our original hypothesis (1) that vagal afferents arising from cardiopulmonary chemo sensors are significantly involved in the initiation of cardiovascular and sympathoinhibitory actions of ANP and that such actions are separate and distinct from those caused by central actions of ANP (22). In view of both the high concentration of such sensors on the epicardial ventricular surface (23, 24) and their demonstrated importance in cardiovascular homeostasis (29), our study focused on the relative importance of epicardial and nonepicardial chemo sensitive vagal afferents in ANP-mediated depressor responses.

Vagal afferents arise from two types of cardiopulmonary end organs: those responsive to mechanical deformation in the ventricular myocardium and those responsive to chemical stimuli (16). Chemosensors are more abundant than mechanosensors and were first described by von Bezold more than 100 years ago (30). Their action potentials are conveyed by small-diameter, unmyelinated (C) fibers within the vagus nerve; they are located primarily near the epicardium, do not fire in synchrony with the arterial pulse, and have no basal discharge (24). They are activated by serotonin (28, 29), bradykinin (12), prostaglandins (19, 27), oxygen-derived free radicals (27), or the 5-HT₃-selective agonist PBG (5). The cardiovascular depressor effects that follow their activation include increased cardiac vagal efferent activity and specific suppression of RSNA (18, 25). These were originally described by Jarisch and Richter (11).

Veelken and colleagues have reported that, in rats, intrapericardial (29) or systemic (28) PBG elicits the typical Bezold-Jarisch response. Either vagotomy or pretreatment with a 5-HT₃ receptor antagonist eliminated the response and demonstrated that the characteristic pattern of hypotension, bradycardia, and inhibition of renal sympathetic outflow can result from activation of pericardial vagal sensors containing serotoninergic receptors. Moreover, they argued that the Bezold-Jarisch reflex was due entirely to activation of pericardial receptors because localized injection of 5-HT₃ antagonist into the pericardial space abolished all depressor effects of systemically injected PBG. Our results (Fig. 2) confirm those of Veelken et al. and demonstrate that in our preparation vagal afferents with 5-HT₃ receptors were available for activation both...
systemically and within the confines of the pericardial space.

Our observation that ANP decreased mean ABP and RSNA in anesthetized rats (Fig. 3) has also been made by others (20). However, involvement of 5-HT_3 receptors in these responses has not been reported previously. The ANP effects shown in Fig. 3 are of the same magnitude as those reported by Schultz et al. (21), but differ from theirs in that we found no significant changes in HR. We have previously reported on the lack of bradycardia after intravenous ANP (3). We believe that the difference in this regard, between our results and those of Schultz, may be attributable to the much smaller dose used here. As in previous experiments, we chose the minimum ANP dose that would yield a 10–20% decrease in mean ABP. Our present results extend previously reported observations by demonstrating that the hypotensive and renal sympathoinhibitory effects of systemically elevated ANP are significantly inhibited when 5-HT_3 receptors are blocked either systemically (Fig. 3; group 4 vs. 3) or pericardially (Fig. 3; group 8 vs. 7). On the other hand, there were neither cardiovascular nor renal sympathetic consequences when ANP was injected and confined to the pericardial space (Fig. 4) where local PBG clearly caused depressor responses (Fig. 4). Although ANP did not directly activate epicardial chemosensors to mediate depressor effects on RSNA, their significant involvement in such responses is one of the interpretations of the observation that pericardially injected 5-HT_3 antagonist was able to attenuate significantly the inhibitory effects of intravenous ANP (Fig. 3; group 8 vs. 7). However, our methods do not rule out the possibility that pericardially injected Ondansetron, a small molecule (molecular weight = 293), may have entered epicardial capillaries and subsequently blocked systemic 5-HT_3 receptors.

An alternative explanation is that intravenously injected ANP can lead to the production of a substance that is capable of reaching epicardial receptors, but that ANP, injected and confined to the pericardial space, cannot produce the substance. Martin (15) has stated that the 5-HT_3 receptor occurs exclusively on central and peripheral neurons. It is a ligand-gated cation channel (15) and therefore causes depolarization. The number of identified endogenous activators of chemosensitive vagal afferents is small. Those relevant to physiological settings are serotonin, prostaglandins, and bradykinin (12, 19, 27, 29). Biochemical paths linking ANP to each of these have not yet been described.

In conclusion, our results demonstrate that both the hypotension and renal sympathoinhibition that follow acute systemic elevation of ANP depend, in part, on activation of 5-HT_3 receptors. Such receptors are present on vagal chemosensory afferents (29), many of which arise from epicardial terminals (24). Direct application of ANP to these terminals within the pericardial space had no effect on blood pressure or RSNA. Although our results do not rule out the possibility that ANP elicits vagal chemosensory reflex effects exclusively via noncardiac afferents, they do suggest the alternative that ANP-mediated excitation of chemosensitive vagal afferents requires a serotonin-like substance that cannot be produced when ANP is confined to the pericardial space.

Perspectives

Serotonergic receptors are located on central and peripheral nerves (15). Therefore, the observations reported here that 5-HT_3 receptors are significantly involved in hypotensive and sympathoinhibitory effects of systemically elevated ANP lend further support to the notion that the cardiovascular effects of the peptide derive partly from chemosensor-initiated reflexes. It is thought that the principal location of the sensory endings is the inferioposterior wall of the left ventricle (14) and that the reflex effects of their activation are involved in the bradycardia of posterior wall infarcts, the hemodynamic patterns of vaso-vagal syncope, and the bradycardia that exacerbates hemorrhagic hypotension. Our results suggest that the sympathoinhibition, hypotension, and occasional bradycardia that have been reported as side effects of acute ANP administration in humans may not be due to direct action of ANP on epicardial chemosensors but require a still unidentified intermediary. If its identity were known then inhibitory cardiovascular side effects of ANP may be prevented while preserving the generally beneficial effects of the peptide on body fluid homeostasis. In view of the small number of agents that are known to excite nonmyelinated vagal afferents, serotonin, bradykinin, and prostaglandins are prospects for future investigation.

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REFERENCES


ANP AND SEROTONERGIC RECEPTORS


