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Type B Atrial Receptor Discharge Increases on Opening a Nonhypotensive Arteriovenous Shunt in the Dog (42027)

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Abstract. A vagovagal cardioacceleratory reflex is activated when an arteriovenous (a-v) shunt is opened in the dog. However, the receptors which initiate this reflex have not been localized. Type B atrial receptor excitation was considered to be a major component of this reflex. The effect of opening a femoral nonhypotensive a-v shunt (i.e., shunt open plus infusion of blood to compensate for the resultant fall in mean arterial and pulse pressure) on type B atrial receptor discharge and heart rate was therefore studied in seven anesthetized, artifically ventilated dogs with β-adrenergic blockade. Right atrial and aortic blood pressures, heart rate, and type B atrial receptor discharge was studied before and after opening a femoral a-v shunt. On opening the a-v shunt there was a significant increase (44%) in the average activity of type B atrial receptors and a small, but significant (6.8%) increase in heart rate. A significant linear positive correlation was observed between the change in activity of type B atrial receptors and the shunt flow. The results suggest that type B atrial receptors may be one of the receptor groups that initiate this vagovagal reflex.

Several studies have established that cardiac acceleration occurs when an arteriovenous (a-v) shunt is opened (1-5). The mechanism underlying such an increase in heart rate was investigated by Gupta and Singh (2) who demonstrated that tachycardia resulting from opening a nonhypotensive a-v shunt (i.e., shunt open plus infusion of blood to compensate for the resultant fall in mean arterial and pulse pressure) was partly due to a cardioacceleratory reflex with both afferent and efferent pathways in each vagus nerve. The present investigation was undertaken to locate the possible receptor sites from which the afferents of such a reflex originate.

The possible receptors are: Type A and type B atrial receptors, ventricular pressure receptors, epicardial receptors, pericardial receptors, and type J pulmonary receptors (6). Except atrial receptors (7), the other receptors have been shown to activate cardioinhibitory reflexes (6, 8-11). It is unlikely that opening an a-v shunt and its consequent effects would result in a decrease in their activity. No conclusive evidence is available of the possible role of type A atrial receptors in mediating reflex heart rate responses (6). Furthermore, there are very few type A atrial receptors in dogs (12, 13). On the other hand type B atrial receptors alter their activity under a number of experimental conditions, and the reflex tachycardia from the atria may be due to activation of type B atrial receptors. The present investigation was therefore undertaken to study the effect of opening a nonhypotensive a-v shunt on type B atrial receptor discharge.

Methods. Experiments were performed on seven adult mongrel dogs of either sex weighing between 11 and 16 kg. The animals were anesthetized with a combination of morphine sulfate, allobarbitone, and urethane to obtain a slow heart rate (14, 15). A cuffed endotracheal tube was inserted and the dog was artificially ventilated at 24 cycles/min with a tidal volume of about 14 ml/kg body wt. Adequate levels of P_aO_2 (>90 mm Hg) were maintained by the addition of humidified oxygen to the inspired air; P_aCO_2 and pH were kept within the ranges of 30-40 mm Hg and 7.3-7.4 pH units, respectively, by adjustment of the stroke volume of the respiratory pump. All such corrections were made before the test period.

Right atrial and aortic blood pressures were measured through catheters inserted into the femoral vessels and connected to Statham strain gauges (P23Db) and recorded on a Grass 7D polygraph. Mean pressures were obtained by electronic damping of the pulsatile signals. Heart rate was calculated from the arterial pressure trace and counted

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over a period of 1 min. The electrocardiogram (EKG) Lead II was amplified (Tektronix 122 preamplifier) and displayed on one channel of an oscilloscope (Tektronix 422).

Nerve fiber recording. Chloride-coated silver electrodes were used to record the action potentials in slips from the vagus nerve in the neck. The technique followed for dissecting the vagus in a paraffin pool was that described by Paintal (16). When a strand was found with fibers firing with a cardiac rhythm it was split up further until a single type B atrial receptor was identified as described by Paintal (17). The nerve impulses were amplified by a Tektronix 122 preamplifier and displayed on the second channel of the oscilloscope. A parallel output through an audioamplifier was used for audiovisual identification of the receptor. Both the EKG and the fiber activity were photographed by a Grass C4 kymograph camera. The fiber response was computed in terms of average discharge rate/sec (the number of spikes counted for about 10 sec in consecutive cardiac cycles divided by time in sec). It was also computed as average discharge rate per cardiac cycle (number of spikes counted over a period of 10 sec for consecutive cardiac cycles divided by number of cardiac cycles).

Preparation of an a–v shunt. An a–v shunt was prepared as described earlier (2). Polyethylene tubings of a wide bore were introduced into the central ends of the right femoral artery and vein. The distal ends of the tubing were connected to a variable speed peristaltic pump (Harvard, Model 1215). The flow through the shunt was measured by a rotameter which was calibrated at the end of the experiment with the animal's own blood. To keep blood from clotting, heparin (Biological Evans) 500 U/kg was injected intravenously before the shunt was set up, followed by 100 U/kg every half an hour. The shunt flow was started by unclamping the femoral artery tubing and simultaneously turning on the peristaltic pump. Blood flowed from the femoral artery into the pump, then through a rotameter, returning to the femoral vein. The speed of the pump was increased over a period of 1 min until the desired flow through the shunt was obtained; the shunt was closed gradually over a period of 1 min.

After the shunt flow was established, blood was infused from a gravity bottle through a catheter in the right external jugular vein. The infusion was given over a period of 1–2 min to raise the mean arterial pressure equal to the control level. For infusion, fresh arterial blood was obtained from a healthy adult mongrel donor dog anesthetized in the same manner as the experimental animal. The dog was heparinized and bled within 20 min prior to the test. The temperature of this blood was adjusted to the rectal temperature of the experimental animal (maintained between 36 and 38°C). The hematocrit of the donor dog was not measured.

Protocol. The protocol for individual experiments was as follows: after a type B atrial fiber was identified, propranolol (Inderal, Imperial Chemical Industries) 1 mg/kg was administered intravenously to the dog 5–6 min prior to the control observation. Effectiveness of the β-adrenergic blockade by propranolol was tested with isoproterenol (Unichem), 2 μg/kg iv. Following a control record of blood pressure, right atrial pressure, EKG, and type B atrial receptor discharge, the shunt was opened over a period of 1 min and a second record taken. Blood was then infused over a period of 1–2 min to raise the mean arterial pressure to a desired level. After 1 min of stabilization another 1-min record was made. The shunt was then closed gradually over a period of 1 min and another record taken. About 1–2 min after closing the shunt, the animal was bled by an amount equal in volume to that infused. In some animals, the test was repeated after about 20–30 min. About 20 min prior to the repeat test, infusion of blood, 5 ml/kg was given to approximately compensate for bleeding from the surgical wound in view of the prior heparinization. Also, a second dose of propranolol 0.5 ml/kg was given 5–6 min prior to the repeat test. The records were then taken on the same fiber following the same protocol. Results from dogs that developed arrhythmias, had very high heart rates, or were physiologically unstable were discarded.

Statistics. Each study variable was analyzed separately. The observations were subjected to analysis of variance (two-way classification). Residuals were also examined to ascertain homogenity of variances and normality. On evidence of significant variations in the

study variables among various treatment schedules, the individual comparisons were done through linear contrasts (18).

Results. The effect of opening an a–v shunt on type B atrial receptor discharge, heart rate, arterial blood pressure, and right atrial pressure was investigated in seven dogs with adequate β-adrenergic blockade. Eleven observations were made on seven single type B atrial fibers.

Figure 1 shows a record from an experiment in which opening an a–v shunt increased the receptor firing rate. In this experiment, type B atrial receptor discharge increased from 12.6 impulses/sec to 28.4 impulses/sec on opening the shunt. Mean arterial pressure decreased by 5 mm Hg, the right atrial pressure increased by 0.5 mm Hg, and heart rate increased from 116 to 125 beats/min. Infusion of blood (50 ml) raised the mean arterial pressure to the control level while the mean right atrial pressure remained unchanged and the heart rate increased to 130 beats/min. The receptor firing showed a small further increase to 29.6 impulses/sec. Closing the shunt promptly decreased the discharge rate to 13.7 impulses/sec and the heart rate to 125 beats/min.

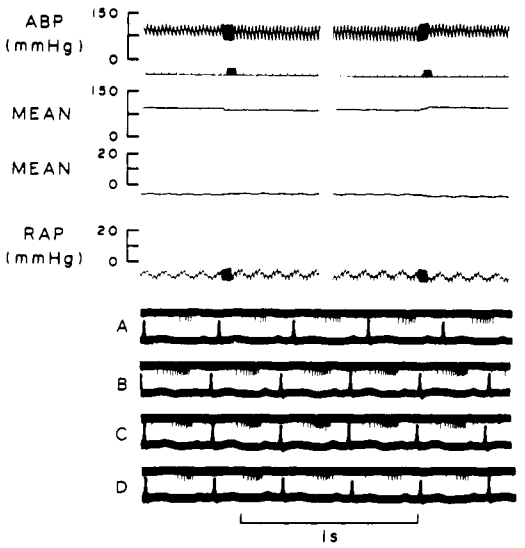

FIG. 1. Experimental recording showing a large increase in type B atrial receptor discharge. Upper record: In each panel from above downward, arterial blood pressure (ABP), time marker (1-sec, 5-sec, 1-min timing marks), mean ABP, right atrial pressure (RAP), mean RAP. Left panel was recorded immediately before opening, during opening, and immediately after opening the shunt. This was followed by infusion of blood. Thereafter, right panel was recorded immediately before closing, during closing, and immediately after closing the shunt. Period of opening or closing the shunt is indicated by 1-sec timing marks superimposed on each other (thick bar). Lower record: shows the receptor discharge. Records A, B, C, and D each show action potentials (upper trace) and ECG (lower trace). A, control; B, shunt open (without infusion of blood); C, shunt open plus infusion of blood; D, shunt closed.

Figure 2 shows an increase in type B atrial receptor discharge on opening the shunt. The receptor discharge increased by 28% from 6.5 to 8.3 impulses/sec, and the heart rate increased from 59 to 79 beats/min on opening the shunt. However, in this experiment the mean arterial pressure increased by 4 mm Hg on opening the shunt, and infusion of blood was therefore not given. The mean right atrial pressure was unchanged. Closing the shunt decreased the discharge rate to 5.3 impulses/sec and the heart rate to 61 beats/min.

The increase in type B atrial receptor discharge was plotted against corresponding shunt flows (Fig. 3). There was a significant ($P < 0.01$, t test, 8 df) linear relationship ($r = 0.823$) between the increase in the impulses/sec and the shunt flow. As shunt flows increased from 40 to 72 ml/kg/min, the number of impulses also increased from control by 1.4–4.6 impulses/sec.

The mean changes in type B atrial receptor discharge, heart rate, mean arterial blood pressure, and mean right atrial pressure are summarized in Table I. Results from one dog in which the blood pressure increased on opening the shunt was not included in the pooled data given in Table I. In general, opening a nonhypotensive a–v shunt resulted in a small but significant 6.8% increase in heart rate ($P < 0.01$, analysis of variance, 24/1 df), while the receptor discharge increased significantly from 12.3 to 17.8 impulses/sec (44% above control, $P < 0.01$, analysis of variance, 23/1 df). The average shunt flow was 59 ml/kg/min. The amount of blood infused to raise the mean arterial pressure equal to or above the control level was 4.9 ml/kg. On closing the shunt the receptor discharge returned to the control level.

Discussion. The increase in heart rate upon

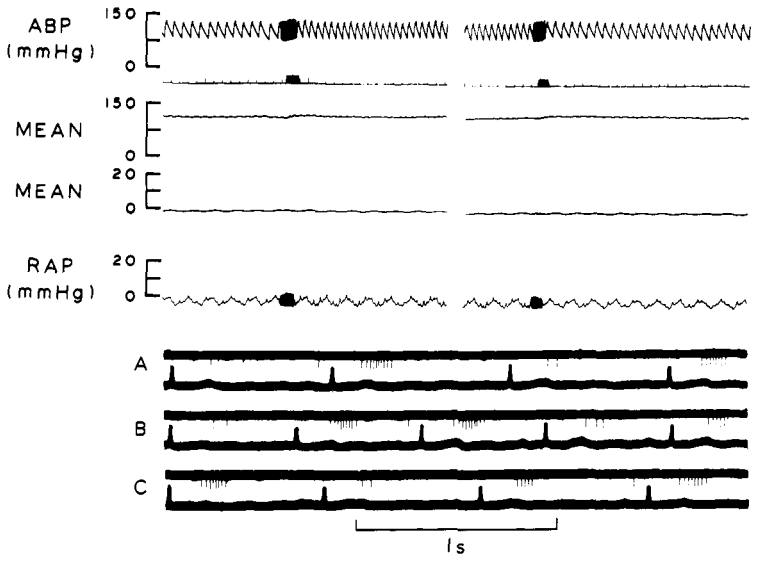

FIG. 2. Experimental recording showing an increase in type B atrial receptor discharge on opening the shunt. In contrast to other experiments the mean arterial pressure increased on opening the shunt in this dog. Upper record: abbreviations as in Fig. 1. In each panel from above downward: ABP, time marker, mean ABP, mean RAP, RAP. Left panel was recorded immediately before opening, during opening, and immediately after opening the shunt. Right panel was recorded immediately before closing, during closing, and immediately after closing the shunt. Lower record: shows the response of a single type B atrial fibre. Records A, B, and C each show action potentials (upper trace) and ECG (lower trace). A, control; B, shunt open (without infusion of blood); C, shunt closed.

opening the shunt in this study was less than that reported earlier (1, 2). The smaller response could be due to the fact that the earlier investigators have reported average control heart rates of 71 and 64 beats/min, respectively, as compared to the average control heart rate of 105 beats/min obtained in our preparations.

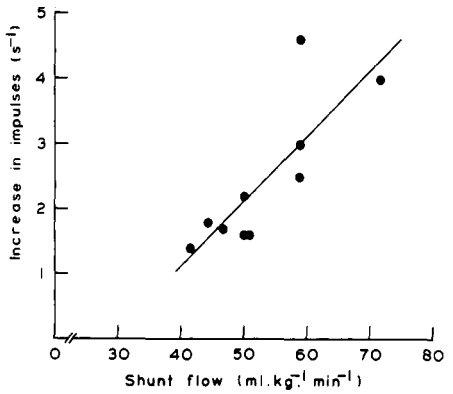

FIG. 3. Increase in type B atrial receptor discharge plotted against shunt flow. The graph shows 10 readings from 6 fibers in 6 dogs. The relation between the two parameters is given by the regression line $y = 0.10143x - 2.9422$ ($r = 0.823$, $P < 0.01$).

The main finding of this study is that opening a nonhypotensive a–v shunt causes a significant increase (44%) in the activity of type B atrial receptors. The linear positive correlation ($r = 0.823$) between the increase in receptor discharge and shunt flow indicates that the increase in activity of type B atrial receptors is due to increase in the venous return resulting from opening the shunt.

In the present study, infusion of blood was given to raise the mean arterial pressure equal to or above the control level. Infusion of blood has been shown to cause an increase in type B atrial receptor discharge (17). However, the increase in receptor firing in the present study was not due to infusion of blood per se because increase in receptor discharge during shunt open plus infusion was not significantly different from shunt open situation ($P < 0.3$, analysis of variance,

TABLE I. EFFECT OF AN A-V SHUNT ON TYPE B ATRIAL RECEPTOR DISCHARGE, HR, MABP, AND MRAP

Group	Impulses/sec	HR[a]	MABP[b]	MRAP[c]
Tests/dogs	9/6	9/6	9/6	9/6
Control	12.34 ± 1.78	104.7 ± 6.8	113.9 ± 5.9	−4.28 ± 0.4
Shunt open				
Change from control	4.12 ± 2.54[f]	11.0 ± 6.0[f]	−2.5 ± 5.9[g]	0.5 ± 0.46[g]
Shunt open plus infusion of blood				
Change from control	5.46 ± 2.67[f]	7.1 ± 5.6[f]	0.7 ± 6.4[h]	0.61 ± 0.44[g]
Shunt closed				
Change from control	0.57 ± 1.88[h]	−6.4 ± 6.6[g]	2.7 ± 6.2[g]	0.11 ± 0.4[h]
VBI[d] 4.9 ± 0.9 (N = 9)				
SF[e] 59 ± 4.3 (N = 9)				

[a] Heart rate (beats/min).
[b] Mean arterial blood pressure (mm Hg).
[c] Mean right atrial pressure (mm Hg).
[d] Volume of blood infused (ml/kg).
[e] Shunt flow (ml/kg/min). Values are means ± SE.
[f] $P < 0.01$.
[g] $P < 0.05$.
[h] Not significant.

23/1 *df*). Additionally, in one dog the mean receptor firing increased even though there was no fall in mean arterial pressure and pulse pressure, and consequently no blood was infused. Furthermore, the effect of slow infusion of 50 ml of blood on receptor discharge was observed in two dogs with shunts closed. In one dog, the receptor discharge remained unchanged (6.7 impulses/sec while in the second dog it showed a small decrease from 11.8 to 10.5 impulses/sec.

In the present study, opening the shunt induced a small change in mean right atrial pressure and the possibility that an increase in atrial type B receptor discharge could occur in the absence of a large increase in atrial pressure, albeit right-sided, needs an explanation. As stated by Paintal (6) the behavior of the type B endings is similar to the behavior of systemic arterial baroreceptors. Thus in both receptors, the adequate stimulus is a pulsatile change, pulse pressure in the baroreceptors and an increase in volume (or amplitude of the v wave) in the atrial receptors. In both receptors, activity tends to increase with mean pressure, but not if the pulsatile stimulus falls simultaneously. In both the frequency of discharge varies with the rate of rise of the stimulus. Opening of an a–v shunt results in a small rise in mean right atrial pressure, but the amplitude of the v wave is increased, the peak of the v wave is higher, the rate of rise of v wave is steeper, and the number of atrial pulsations (on account of the tachycardia response) is increased (Fig. 3 in Ref. 2). These changes could result in an increase in the activity of type B receptors in spite of minimal rise in mean atrial pressure.

No attempt was made to localize the site (whether right or left) of origin of the type B fibers and therefore it is not possible to correlate the changes in receptor discharge to changes in the atrial pressure. However, it is unlikely that changes of pressure in the left atrium would be qualitatively different from those induced on the right side. It is even more unlikely that changes of pressure in the left atrium would be smaller than those in the right atrium.

Gupta and Singh (2) reported that tachycardia resulting from opening a nonhypotensive a–v shunt was due to a reflex with its afferent pathway in the vagus nerve. They also studied single fiber aortic and carotid baroreceptor discharge during opening the shunt and found that heart rate changes resulting from opening the shunt (with or without infusion of blood) were not related to control heart rate or to changes in mean right atrial or arterial pressure. The results in the present study indicate that type B atrial

receptors could be one of the receptor groups mediating the tachycardia on opening the shunt. However, opening an a–v shunt is a diffuse stimulus which can cause an increase in receptor activity at several sites.

In conclusion, this study shows that non-hypotensive opening of an a–v shunt results in a significant increase in activity of type B atrial receptors. It is likely that in a–v shunts type B atrial receptors are one of the receptor groups which cause cardioacceleration by a vagovagal mechanism.

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1. Coleridge JCG, Linden RJ. The effect upon the heart rate of increasing the venous return by opening an arteriovenous fistula in the anaesthetized dog. J Physiol (London) **130**:674–702, 1955.
2. Gupta PD, Singh M. Neural mechanism underlying tachycardia induced by non-hypotensive a–v shunt. Amer J Physiol **236**:H35–H41, 1979.
3. Guyton AC, Sagawa K. Compensations of cardiac output and other circulatory functions in areflex dogs with large A–V fistulas. Amer J Physiol **200**: 1157–1163, 1961.
4. Nakano J, Schryver CDe. Effects of arteriovenous fistula on systemic and pulmonary circulations. Amer J Physiol **207**:1319–1324, 1964.
5. Van Loo A, Hemingway EC. Circulatory changes in the dog produced by acute arteriovenous fistula. Amer J Physiol **158**:103–112, 1949.
6. Paintal AS. Vagal sensory receptors and their reflex effects. Physiol Rev **53**:159–227, 1973.
7. Burkhart SM, Ledsome JR. The response to distension of the pulmonary vein-left atrial junctions in dogs with spinal section. J Physiol (London) **237**: 685–700, 1974.
8. Arndt JO, Brambring P, Hindorf K, Röhnelt M. The afferent impulse traffic from atrial A-type receptors in cats. Does the A-type receptor signal heart rate? Pflüegers Arch **326**:300–315, 1971.
9. Salisbury PE, Cross CE, Rieben PA. Reflex effects of left ventricular distension. Circ Res **8**:530–534, 1960.
10. Sleight P, Widdicombe JG. Action potentials in afferent fibres from pericardial mechanoreceptors in the dog. J Physiol (London) **181**:259–269, 1965.
11. Thorén P. Evidence for a depressor reflex elicited from left ventricular receptors during occlusion of one coronary artery in the cat. Acta Physiol Scand **88**:23–34, 1973.
12. Coleridge JCG, Hemingway A, Holmes RL, Linden RJ. The location of atrial receptors in the dog: A physiological and histological study. J Physiol (London) **136**:174–197, 1957.
13. Kappagoda CT, Linden RJ, Mary DASG. Atrial receptors in the dog and rabbit. J Physiol (*London*) **272**:799–815, 1977.
14. Coleridge JCG, Linden RJ. The effect of intravenous infusion upon the heart rate of the anaesthetized dog. J Physiol (London) **128**:310–319, 1955.
15. Gupta PD. Spinal autonomic afferents in elicitation of tachycardia in volume infusion in the dog. Amer J Physiol **229**:303–308, 1975.
16. Paintal AS. The conduction velocities of respiratory and cardiovascular afferent fibres in the vagus nerve. J Physiol (London) **121**:341–359, 1953.
17. Paintal AS. A study of right and left atrial receptors. J Physiol (London) **120**:596–610, 1953.
18. Armitage P. Comparison of several groups. In: Statistical Methods in Medical Research. London, Blackwell, Ch 7, pp203–204, 1974.

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