Reflex Cardiovascular and Respiratory Effects of Serotonin in Conscious and Anesthetized Dogs

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SUMMARY  Cardiovascular and respiratory effects of intra-left atrial or intra-left ventricular injection of serotonin were studied in conscious dogs (n = 8), anesthetized closed-chest dogs (n = 13) and anesthetized open-chest dogs (n = 9). Serotonin (50-200 μg), injected as a bolus, resulted in an initial bradycardia and hypotension followed by a delayed tachycardia and hypertension in the conscious dogs. The hypertension was seen as an increase of 21.5 ± 2.7 (mean ± SE) mm Hg from a control pressure of 102.5 ± 1.9 mm Hg, whereas the initial decrease in pressure was 22.6 ± 1.9 mm Hg. The tachycardia was 23.3 ± 3.9 beats/min above a control heart rate of 104.9 ± 3.9 beats/min whereas the bradycardia was 58.5 ± 3.7 beats/min below control. There was a significant attenuation of the hypotension in both groups of anesthetized dogs. In fact, no hypotension was elicited in the open-chest anesthetized dogs. Open-chest anesthetized dogs showed only a hypertensive response (mean increase 67.2 ± 5.5 mm Hg). Stimulation of respiration was seen in all groups of dogs. In conscious dogs there was a 214.8 ± 15.4% increase in respiratory depth and a 20.8 ± 3.1 breaths/min increase in respiratory rate. Atropine significantly reduced the bradycardia and abolished the hypotension in conscious dogs. Bilateral cervical vagotomy did not abolish the response in open-chest anesthetized dogs. We conclude that the so-called "hypertensive coronary chemoreflex" is altered dramatically by the state of the preparation and by anesthesia. Circ Res 47: 509–515, 1980

A HYPERTENSIVE coronary chemoreflex has been described (James et al., 1975; James et al., 1976), which is characterized by an intense vasoconstriction, resulting in some cases in elevations of arterial pressure of over 100 mm Hg within a few seconds. In addition, a transient bradycardia (James et al., 1975), hyperpnea (Eckstein et al., 1971), and depression of atrial contractility (Urthaler et al., 1978) have been described. The stimulus for activation of this reflex is serotonin, which must be injected close to the left coronary ostia to be effective. If serotonin is given into the descending thoracic aorta or carotid circulation, it is ineffective in eliciting this reflex (James et al., 1975). If it is given into the distal two-thirds of the left coronary system approximately 1-1.5 cm distal to the origin of the main left coronary, it results in a slight Bezold-Jarisch response (i.e., bradycardia and hypotension) (James et al., 1975). The serotonin-sensitive receptor is a small area of chemoreceptor-like tissue which is supplied by a small coronary artery arising from either the left circumflex or the left anterior descending coronary artery near the origin (Eckstein et al., 1971). This tissue has been observed in dogs, cats, and humans (James et al., 1975). The afferent limb of this hypertensive reflex is reported to be in the vagi and the efferent limbs are in both the vagi and cardiac sympathetics as well as the peripheral sympathetic nerves (James et al., 1975; Urthaler et al., 1978; Hageman et al., 1978). It has been proposed that this reflex may be beneficial to humans when a thrombus accumulates in the left main coronary artery (James et al., 1976). The platelets, which are rich in serotonin, would provide the stimulus to this chemoreceptor-like tissue causing a rapid elevation in arterial pressure, thereby dislodging the clot.

With the exception of three conscious dogs (James et al., 1975), all of the work concerning this reflex was done in the open-chest anesthetized dog. Little attention has been given to the role of the respiratory response in mediating the vascular components of this reflex. The present study was undertaken to investigate the various components of this reflex in the conscious dog, in the closed-chest anesthetized dog, and in the open-chest anesthetized dog.

Methods

Thirty mongrel dogs weighing 22.7 ± 0.9 (mean ± SE) kg were divided into three groups as follows: 8 conscious dogs, 13 anesthetized closed-chest dogs, and 9 anesthetized open-chest dogs.

Conscious Dogs

A left thoracotomy was done at least 2 weeks prior to the experiment to implant Silastic catheters in the thoracic aorta and in either the left ventricle or left atrium. Four dogs also had a Silastic catheter

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implanted in the left circumflex coronary artery approximately 1–1.5 cm distal to its origin from the main left coronary artery. During the recovery phase, the dogs were trained to stand quietly in a Pavlov sling.

Atropine sulfate (0.2 mg/kg) was given to six of the dogs. Effectiveness of the blockade was assessed by inhibition of the bradycardia and hypotension elicited by a 50-µg iv bolus of acetylcholine. Ventilation was measured with a pneumograph placed around the chest and attached to a Millar catheter-tipped micromanometer.

**Anesthetized Dogs**

Dogs were anesthetized with either pentobarbital sodium (Diabutal, Diamond Labs, 30 mg/kg) or α-chloralose (Fischer, 100 mg/kg). In closed-chest anesthetized dogs, a catheter was placed in the left ventricle via a femoral artery. In open-chest anesthetized dogs, a catheter was placed in the left atrium through a small pulmonary vein. Arterial pressure was measured from the arch of the aorta with a Millar catheter-tipped micromanometer inserted through a femoral artery. A femoral venous catheter was placed for administration of supplemental anesthesia. Temperature was controlled at 37°C, with a heating pad, with the aid of a Yellow Springs Temperature Controller. Arterial blood gases were similar to those observed in the conscious dogs described above and were maintained within those limits. In several anesthetized dogs, the cervical vagi were isolated and loose ligatures placed around them for later retrieval and sectioning. Renal nerve efferent activity was recorded in six open-chest anesthetized dogs by means of bipolar platinum-iridium electrodes. The signal was differentially amplified with a Grass P15 preamplifier and displayed on a chart recorder along with integrated nerve activity. The nerve traffic also was displayed on a storage oscilloscope. Renal nerve activity was quantified by a "RC" integrator with a time constant of 3.84 seconds. Zero renal nerve activity was obtained at the end of the experiment by crushing the central end of the nerve. In three of the six dogs, renal nerve activity was recorded after bilateral cervical vagotomy. Integrated renal nerve activity was quantified by pen deflection and expressed as percent change from control.

Ventilation was monitored with a pneumograph, placed around the chest in closed-chest anesthetized dogs and at the level of the diaphragm in open-chest anesthetized dogs. Respiratory depth was quantified by pen deflection and expressed as the percent change from control. Heart rate was measured in all dogs with a Honeywell cardiotachometer, which was triggered by the arterial pulse. The maximum bradycardia and tachycardia following the injection of serotonin was used to quantify the changes in heart rate. Mean arterial pressure was measured by electronic damping of the arterial pulse. All parameters were recorded permanently on magnetic tape (Vetter; model D) and on a Hewlett-Packard eight-channel recorder (model 7758A).

**Experimental Protocol**

After control measurements of blood pressure, heart rate, respiration, and (in some dogs) renal nerve activity had been made, a bolus of serotonin (creatinine sulfate complex; Sigma) was injected into the left ventricle or the left atrium. The serotonin was dissolved in isotonic saline and given in doses ranging from 50 to 200 µg. The concentration was 100 µg/ml; therefore the volume of injectate varied from 0.5 to 2.0 ml. Injection was done over a 1- to 2-second period. More than one injection usually was given each dog. Each injection was separated by an interval of 2–5 minutes. No tachyphylaxis was observed during this protocol. There was no difference in responses elicited by the intraventricular or by the intra-atrial route. Similar injections of the serotonin vehicle (isotonic saline) were also done in each group of dogs. The peak response of each parameter following the injection of serotonin was recorded (initial and delayed).

The data among groups were analyzed for statistical significance using a one-way analysis of variance and Duncan’s multiple range test. The data within a group were analyzed by the paired t-test.

**Results**

Figure 1 shows representative recordings from one dog in each group. Serotonin (200 µg) was injected at the arrow in each panel. The characteristic response in the conscious dog, as well as in the closed-chest anesthetized dog (panels A and B), is an abrupt bradycardia followed by a slight tachycardia. There is an initial hypotension which coincides with the bradycardia. Although not seen in Figure 1B, in most dogs we saw a secondary hypertension which coincided in time with a secondary tachycardia. Simultaneously with the vascular effects, or in some dogs slightly before, there is a stimulation of respiration. The response that was observed in the open-chest anesthetized dog (panel C) was different from that seen in the previous two groups. Although the dog could not alter its ventilation, it made strong respiratory movements, as can be seen in Figure 1C. There was an immediate pressor response in the open-chest anesthetized dog (latency 3.4 ± 0.3 sec) in which blood pressure rose to a maximum of 185 ± 61 mm Hg from a control pressure of 117 ± 36 mm Hg in 12.0 ± 0.7 seconds. The initial hypotension seen in conscious and closed-chest anesthetized dogs was absent. The bradycardia was present (and, in some dogs, prolonged), probably due to the marked hypertension. There was no significant change in blood pressure, heart rate, or respiration when the vehicle (isotonic saline) was given by either the intra-atrial or intraventricular route to seven conscious and nine anesthetized dogs.

Figure 2 shows the mean change from control of...
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FIGURE 1 The response to serotonin injected into the left atrium or left ventricle in A, a conscious dog; B, a closed-chest anesthetized dog; and C, an open-chest anesthetized dog. Serotonin (200 μg) was injected as a bolus at the arrows. The respiratory tracing shows inspiration as a downward deflection in A and as an upward deflection in B. The respiratory tracing in C depicts an inspiratory effort; * symbols indicate statistical significance.

blood pressure and heart rate in the three groups of dogs after the injection of serotonin. When compared to the conscious dogs, the initial hypotension was significantly decreased in the anesthetized closed-chest dogs and was abolished in the anesthetized open-chest dogs. Only a hypertensive response was seen in the open-chest anesthetized dogs. This is depicted as an increase in pressure in the left-hand panel of Figure 2. Likewise, the initial bradycardia was reduced in the closed-chest and open-chest anesthetized dogs relative to the response in the conscious dogs. The hypertensive responses were greater in the two groups of anesthetized dogs, compared to those in the conscious animals. The

FIGURE 2 The mean changes in blood pressure and heart rate in the three groups of dogs studied following serotonin injection. The bars on the left of each panel show the mean (± SE) hypotensive and bradycardiac response. (These bars refer to the initial or immediate responses; see text.) The bars on the right show the hypertensive, tachycardiac response for each group. (These bars show the delayed responses; see text.) Control mean arterial pressures and control heart rates for both hypo- and hypertensive responses are shown below each bar on the left of each panel. The numbers in parentheses in each bar represent the number of injections made in that group.
CONSCIOUS (n=9)

FIGURE 3 The mean percent changes in respiratory depth and the mean change in respiratory rate for conscious and anesthetized closed-chest dogs following injection of serotonin. Since respiratory depth was measured in units of pen deflection, no control values have been provided in the figure. The control rates are shown for respiratory rate below the bars. Numbers in parentheses denote the number of injections upon which the data are based.

latency for onset of the hypertension in the open-chest anesthetized dogs was the same as the latency of the hypotensive response in the conscious and closed-chest anesthetized dogs (3.2 ± 0.2 and 3.4 ± 0.5 seconds, respectively). The secondary tachycardia was significantly greater in the open-chest anesthetized dogs. This secondary tachycardia was not seen in Figure 1C because of the large hypertensive response which evoked a bradycardia. As can be seen from Figure 2, the control heart rates and blood pressures were significantly higher in both groups of anesthetized dogs than in the conscious dogs.

Figure 3 shows the mean data for the respiratory response in eight conscious and nine closed-chest anesthetized dogs. Following the injection of serotonin there was a 214.8 ± 15.4% increase in respiratory depth in the conscious dogs, whereas there was a 122.7 ± 19.6% increase in respiratory depth in the closed-chest anesthetized dogs, a significantly lower value. Respiratory rate increased by 20.8 ± 3.1 breaths/min in the conscious dogs. A similar increase of 25.0 ± 3.1 breaths/min was observed in the anesthetized closed-chest dogs. These changes in rate were not significantly different from each other. The absolute respiratory rate in the anesthetized closed-chest dogs was significantly lower than in the conscious dogs (P < 0.001), probably because of the depressant effects of the anesthesia. In addition, the somewhat elevated respiratory rate in the conscious dogs may have been due to panting in some animals.

We concur with previous workers that the receptor that initiates the reflex effects of intra-left atrial or left ventricular serotonin must be located near the proximal left coronary system (Eckstein et al., 1971; Eckstein, 1977; James et al., 1975). In four conscious dogs with chronic left circumflex coronary artery catheters, we injected 200 μg of serotonin into the coronary approximately 1–1.5 cm from the main left coronary artery. Arterial blood pressure decreased by 12.8 ± 5.7 mm Hg (P < 0.05), and heart rate increased by 1.7 ± 6.2 beats/min. There was no stimulation of respiration following intracoronary injection. Intracarotid injections (done only in anesthetized dogs) and intra-aortic injections (given approximately 5 cm from the aortic valve in conscious dogs) did not result in any significant cardiovascular or respiratory responses.

The efferent component of the bradycardia and hypotension was investigated in six of the conscious dogs. Figure 4 shows that, after the administration of atropine the bradycardia was significantly reduced and the initial hypotension was abolished completely. Although the secondary hypertension was not altered by atropine, the tachycardia was reduced significantly.

The effect of vagal section on the cardiovascular response to serotonin in five open-chest anesthetized dogs.
tized dogs can be seen in Figure 5. Right vagotomy did not significantly alter the hypertension or the tachycardia. However, a combination of right and left vagotomy, significantly reduced the magnitude of the hypertensive response but did not alter the tachycardia. We could not abolish completely the hypertensive response to serotonin (by bilateral vagotomy) in any open-chest anesthetized dog.

In open-chest anesthetized dogs, the efferent component of the hypertension was investigated by recording renal efferent nerve activity. Figure 6 shows a record from one such dog. After the left atrial injection of 200 μg of serotonin (at arrow), there is an almost immediate increase in renal nerve activity followed by respiratory movements and the hypertensive response. Renal nerve activity then returns to control after about 15 seconds. Table 1 shows the percent increase in renal nerve activity in this group of dogs before and after section of the right and left vagus. There was a 234.5 ± 70.0% increase in renal nerve activity following the administration of serotonin in the intact open-chest anesthetized dog (P < 0.01). Right vagotomy did not alter the response. Bilateral vagotomy reduced the increase in renal nerve activity below that of the intact and right-vagotomized dogs; however, this value was not statistically significant from the intact dogs, probably due to the small number of dogs and the high standard error, but clearly the increase in renal nerve activity could not be abolished completely by bilateral vagotomy.

**Discussion**

A reflex alteration in blood pressure and heart rate has been demonstrated following the intra-atrial or intraventricular injection of serotonin. The characteristics of this response are dramatically altered by the experimental preparation. In the open-chest, pentobarbital- or chloralose-anesthetized dog, this reflex was characterized by a rapidly developing hypertension and an initial bradycardia. These responses were similar to those described by James et al. (James et al., 1975; James et al., 1976; Hageman et al., 1977; Urthaler et al., 1978). In the present experiments, the cardiovascular response was dramatically altered when serotonin was injected into the left atrium or left ventricle of conscious dogs and of closed-chest anesthetized dogs. In these two groups, it was characterized primarily by a bradycardia and hypotension. James et al. (1975) have injected serotonin into the left atrium of three conscious dogs and observed a transient hypotension, followed by a hypertension (which ranged from 48 to 73 mm Hg above control), as well as a transient bradycardia. This hypertensive response would appear to be lower than the mean for their 32 open-chest anesthetized dogs, which was 96 ± 18 mm Hg above control.

In all of the dogs in the present study, the administration of serotonin by the intra-atrial or intraventricular route resulted in an immediate and profound stimulation of both the depth and rate of respiration. Indeed, even in our open-chest anesthetized dogs, we observed powerful respiratory movements following the serotonin injection. The only reference in the literature to the respiratory components in this reflex is the work of Eckstein et al.
(1971) who observed inspiratory efforts in 18 of 20 animals when serotonin was injected at or close to the coronary vessels supplying the receptor tissue. Prior to the present study, the respiratory response has been largely ignored in describing this reflex.

The efferent component of the reflex bradycardia and hypotension in conscious dogs appears to be located primarily in the vagus, since the administration of atropine completely abolished the hypotension and significantly reduced the bradycardia. Alternately, it is possible that there is a peripheral sympathetic cholinergic component mediating the hypotension in the conscious dog which would be blocked by atropine. The secondary tachycardia also was reduced after atropine, most likely because no hypotension was elicited. This would be consistent with the view that the delayed tachycardia is mediated by baroreceptor unloading. Since some bradycardia still was elicited by serotonin after administration of atropine, some sympathetic withdrawal may also be a component of the bradycardia in the intact dog. Although we observed a potent activation of the afferent renal sympathetic nerves in the open-chest anesthetized dogs after administration of serotonin (Fig. 6), it is unclear whether this occurs in the conscious dog. This sympathetic stimulation undoubtedly results in the systemic hypertension seen in the open-chest anesthetized dog (James et al., 1980). If this sympathetic stimulation were a generalized phenomenon and took place in the conscious dog following serotonin administration, one would expect the hypertension to be present, at least in part, when serotonin was given following atropine. In our experiments, this was not the case. Therefore, it can be inferred that there was either no or a greatly reduced activation of the peripheral sympathetic nervous system in the conscious dog compared to the open-chest anesthetized dog. In the open-chest anesthetized dog, atropine abolishes the negative inotropic effect on the atria that is elicited by serotonin, as well as the bradycardia, but does not abolish the hypertension (Urthaler et al., 1978). This further points to a real difference in this reflex when elicited in a conscious intact preparation or an anesthetized open-chest preparation.

The present study indicates that the afferent arm of this reflex is not exclusively in the vagus, since we could not abolish the hypertension or the respiratory response by bilateral cervical vagotomy in the open-chest anesthetized dogs. It would appear, however, that at least part of the afferent pathway is in the left vagus since, after left vagotomy, we could reduce the hypertension significantly. Urthaler et al. (1978) came to the conclusion that the afferent pathway was exclusively in the vagus since bilateral stellectomy did not abolish the reflex whereas vagotomy did (James et al., 1975). However, Hageman et al. (1978) could not eliminate the reflex in 16 of 39 dogs following various combinations of vagal and cardiac nerve cooling. Although James et al. (1975) observed a depressed hypertensive response after bilateral vagotomy in 12 dogs, they could not abolish the hypertension. There was still an increase of 17 ± 6 mm Hg after bilateral vagotomy compared to 88 ± 15 mm Hg before vagotomy. More recent evidence indicates that the residual effect may be mediated by the action of serotonin on the adrenal medulla, although latency data would have been helpful to elucidate this. Urthaler et al. (1978), in a more recent paper, state "...severing the vagi in the neck (with or without bilateral stellectomy) completely abolished the vasoconstriction." We cannot find any evidence for this statement in their paper. Furthermore, there are sympathetic afferent pathways which do not go through the stellate (Mizeres, 1955). Based on our findings, we conclude that part of the afferent pathways for this reflex traverse the sympathetic nerves and do not travel via the vagi. Sympathetic afferents have been shown to convey mechanical and chemical stimuli into the central nervous system (CNS) (Ueda et al., 1969; Uchida and Murao, 1975; Uchida and Murao, 1973). Indeed, there are several pieces of evidence that indicate that cardiac sympathetic afferents convey cardiac pain to the CNS (Brown, 1968; White et al., 1933).

It is not clear why we found such a dramatic
alteration in the response to serotonin when we compared conscious dogs with open-chest anesthetized dogs. Anesthesia itself has been shown to alter the response to a variety of drugs as well as the reflex control of blood pressure, myocardial contractility, and peripheral flow (Vatner and Braunwald, 1975). Surgical trauma, especially in the open-chest dog, may alter cardiovascular reflex mechanisms by decreasing heart size and cardiac output and by altering resting vagal tone. This, in turn, could lead to a variety of stimuli from both mechanoreceptors, chemoreceptors, and nociceptors which, when processed by the CNS, can alter the response of a given reflex such as the one described here.

In summary, we have compared the response to intra-atrial or intra-ventricular serotonin in three groups of dogs: conscious, anesthetized closed-chest, and anesthetized open-chest. It is clear that the experimental preparation can alter the response elicited dramatically. Because of the profound bradycardia and hypotension that we observed in the conscious dog, we would not term this response a hypertensive chemoreflex. In addition, one should take great care in extrapolating the clinical significance of this reflex to the intact awake human. The hypertensive response may be elicited, however, in humans undergoing thoracic surgery under the influence of general anesthesia.

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