Chemical stimulation of cardiac receptors attenuates locomotion in mesencephalic cats

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Pickar, J o e l G. Chemical stimulation of cardiac receptors attenuates locomotion in mesencephalic cats. J. Appl. Physiol. 83(1): 113–119, 1997.—The purpose of the present investigation was to determine whether chemical stimulation of cardiac receptors is sufficient to inhibit locomotion. Decerebrate, unanesthetized cats were induced to walk on a treadmill by electrically stimulating the mesencephalic locomotor region (MLR). Cardiac receptors were stimulated by injecting nicotine (62.3 ± 8.6 µg/kg, mean ± SE) into the pericardial sac. Cardiac nerve activity was reversibly blocked by injecting procaine (2%) into the pericardial sac. Locomotion was monitored using bipolar needle electrodes inserted into the lateral gastrocnemius (LG) and tibialis anterior (TA) muscles. Integrated electromyographic (IEMG) activity from each muscle was quantified on a step-by-step basis. Intrapericardial (ipc) nicotine inhibited locomotion and evoked the coronary chemoreflex. Blood pressure and heart rate decreased significantly by 45.6 ± 7.1 mmHg and 59.3 ± 12.3 beats/min, respectively. Nicotine ipc significantly reduced IEMG activity by 24–28% in the LG muscles. The TA muscles were not affected consistently by ipc nicotine. The locomotor inhibition and the depressor reflex paralleled each other and occurred within 5 s of nicotine injection. Procaine ipc blocked the nicotine-induced locomotor inhibition and depressor reflex. The effects of procaine were largely reversible, because ipc nicotine reduced IEMG activity in the LG (25–46%) but not in the TA muscles after washing procaine from the pericardial sac. These results demonstrate that cardiac receptors sensitive to nicotine inhibit MLR-induced locomotion in the decerebrate cat. These findings indicate the presence of a neural pathway from the heart whereby endogenous stimuli could reflexly alter motor control.

coronary chemoreflex; exercise; viscerosomatic reflex; nicotine; somatomotor inhibition; epicardial receptors

CHEMICAL STIMULATION of visceral afferents from the heart and lung evokes the well-known coronary chemoreflex and pulmonary depressor reflexes, respectively (8). These reflexes consist of bradycardia, hypotension, and apnea (7, 8, 21). Visceral afferents from the cardiopulmonary region also evoke somatic responses, consisting of an inhibition of somatomotor activity in the cat and rat. For example, activation of vagal C fibers by using intravenous nicotine or phenyl biguanide inhibits monosynaptic and polysynaptic muscle reflexes in the cat (9, 12, 14). In addition, a more complex motor behavior, namely locomotion, is also inhibited by intravenous phenyl biguanide and phenyl biguanide in the cat and rat (10, 15, 24).

The contribution of the heart alone to the inhibition of locomotion has not been determined in the cat or the rat. Intravenous and left ventricular injection of phenyl biguanide inhibits locomotion in decerebrate cats, suggesting that both pulmonary receptors and cardiac receptors could contribute to the inhibition (24). Right atrial or femoral venous injection of phenyl biguanide also inhibits locomotion in the conscious cat (15) and rat (10), but left-sided vs. right-sided injections (with respect to the heart) have not been compared in these conscious preparations. Increasing left atrial pressure by using an inflatable balloon placed in the left atrium, a mechanical stimulus that potentially excites pulmonary and atrial receptors (6, 16), also inhibits locomotion in decerebrate cats (24). In the conscious rabbit, however, chemical stimulation of the heart does not inhibit locomotion (19).

The purpose of the present investigation was to determine whether chemical stimulation of cardiac receptors alone is sufficient to inhibit locomotion in the decerebrate cat. Nicotine was used because it has previously been shown to stimulate vagal C fibers from the heart (8, 18, 29). Phenyl biguanide was not used because it also stimulates sympathetic fibers from the heart (3). Afferent input traveling in the vagi and sympathetic nerves from the heart can occlude each other in the central nervous system (31). The hypothesis was tested that nicotine injected into the pericardial sac inhibits locomotion in the decerebrate cat.

METHODS

General Procedures

Studies were performed on 13 cats weighing 2.4–3.5 kg. All experiments were performed in accordance with the Guiding Principles in the Care and Use of Animals approved by the American Physiological Society. Anesthesia was induced by using a mixture of O2 (5 l/min) and halothane (5%) delivered to a sealed plastic chamber. Anesthesia was maintained by delivering O2 (2 l/min) and 3% halothane through a face mask placed over the cat’s nose and mouth. Catheters were placed in a common carotid artery and an external jugular vein to monitor blood pressure and to introduce fluids, respectively. The trachea was intubated, and the cat was maintained on O2 and 1.5–3% halothane. Arterial pH, Pco2 and PO2 were monitored by using a pH/blood-gas analyzer (Ciba-Corning 238). Arterial blood-gas values were maintained within the normal range (pH 7.32–7.43; Pco2 32–35 Torr; and PO2 > 85 Torr). Arterial pH, Pco2 and PO2 were corrected by injecting sodium bicarbonate (10%), by mechanically ventilating the lungs (model 661 respirator, Harvard Apparatus), and by supplementing the inspired gas with O2. Each cat was given dexamethasone (4 mg iv) to reduce tissue swelling.

An incision was made through the fifth intercostal space, and the pericardial sac was exposed. The surface of the heart was superfused with saline, nicotine, or procaine (see Protocols) via a Silastic cannula inserted through a small hole placed in the pericardial sac. The hole was sealed around the cannula by using a purse-string suture and tissue cement (Loctite, Cleveland, OH). The absence of leakage from the pericardial sac was confirmed at the end of each experiment.
by injecting a volume of saline at least equal to the volume used during the experiment and large enough to cause ballooning of the pericardial sac.

The procedures for decerebration and induction of locomotion have been described in detail elsewhere (23, 24) and are presented here only briefly. The cat was placed in a stereotaxic unit and suspended over a treadmill. A precollicular-postmammillary decerebration was performed; gaseous anesthesia was then discontinued. Locomotion on the moving treadmill was evoked by using a monopolar stimulating electrode (20–40 Hz, 0.5–1.0 ms, <120 µA) inserted into the mesencephalic locomotor region (MLR; Horsley-Clarke coordinates P2, L4 or R4, H0). Rhythmic muscle activity was monitored by using bipolar needle electrodes inserted bilaterally into paired hindlimb flexor [tibialis anterior (TA)] and extensor [lateral gastrocnemius (LG)] muscles. Electromyographic (EMG) signals were amplified (×1,000; band pass, 0.01–3 kHz), full-wave rectified, and integrated (time constant = 100 ms). Decerebrate cats entrain their stepping frequency to the speed of the treadmill (28). The treadmill speed was set by using a tachometer to yield a stepping frequency of not less than 1 step/s. Speed was set by using a tachometer to yield a stepping frequency to the speed of the treadmill (28). The treadmill was loaded with saline or nicotine and cleared with saline after each protocol of an experiment were isovolumetric (range 2–2.5 ml). Injectates were withdrawn at the onset of locomotion. Procaine was quickly withdrawn at the onset of locomotion. Pain sensitive cardiac receptors (18, 29). In this fashion stimulates chemosensitive and possibly mechanosensitive cardiac receptors (18, 29).

The magnitude of the nicotine-induced bradycardia decreased (P < 0.05), heart rate significantly decreased heart rate (−59.3 ± 12.3 beats/min; n = 13) and blood pressure decreased (−45.6 ± 7.1 mmHg; n = 13). Procaine ipc blocked the nicotine-induced depressor response; blood pressure decreased only −1.9 ± 2.1 mmHg and heart rate increased 1.0 ± 0.7 beats/min, respectively, in seven cats. Washing procaine from the pericardial sac reversed the nicotine-induced depressor response; heart rate significantly decreased (−56.4 ± 13.2 beats/min) and blood pressure decreased (−36.0 ± 14.8 mmHg; P < 0.08) in five cats. The magnitude of the nicotine-induced bradycardia and, to a lesser extent, the nicotine-induced hypotension were similar before procaine and after washing procaine from the pericardial sac.

Visual inspection of individual records and of locomotion on the treadmill showed that ipc nicotine inhibited locomotion. iEMG activity was attenuated in at least one muscle in 7 of the 13 cats and completely abolished in LG and TA muscles in 4 of the 13 cats. In two cats, there was no change in iEMG activity after ipc nicotine. A representative record from one cat is shown in Fig. 2. Saline ipc did not evoke a cardiovascular response, nor did it have an effect on iEMG activity (Fig. 2A). Nicotine ipc evoked a depressor response and attenuated iEMG activity in the LG but not the TA muscles (Fig. 2B). The attenuation of iEMG activity began as
the cannula was cleared of nicotine and paralleled the change in blood pressure and heart rate.

Figure 3 shows group data of step-by-step changes in iEMG activity during the four experimental protocols. Saline ipc had little effect on iEMG activity in the LG (Fig. 3A) or TA (Fig. 3B) muscles. Nicotine ipc attenuated iEMG activity in the extensor muscles (Fig. 3A) and to a lesser extent in the flexor muscles (Fig. 3B). Mean iEMG activity in the left and right LG muscles was reduced significantly by 24 and 28%, respectively, after ipc nicotine compared with the mean iEMG activity of the same muscle after ipc saline (Table 1) in 13 cats. Although mean iEMG activity in the left and right TA muscles was reduced by 14 and 7%, respectively, after ipc nicotine compared with the mean iEMG activity of the same muscle after ipc saline, the decrease was not significant. Mean iEMG activity in the right but not the left LG muscle after ipc nicotine was reduced significantly compared with both the ipsilateral and contralateral TA muscles after the same protocol (Table 1). The onset of locomotor inhibition began within eight steps (~5 s, see Fig. 2B) of clearing the intrapericardial cannula of nicotine (Fig. 3A).

Procaine blockade abolished the nicotine-induced inhibition of locomotion in the seven cats tested (Fig. 3, A and B), because the mean iEMG activities in the LG and TA muscles were not different from those after ipc saline (Table 1). Mean iEMG activity was lower in the left LG compared with the right LG muscle after ipc nicotine during procaine blockade. After washout of procaine from the pericardial sac, the nicotine-induced inhibition of locomotion was restored in the five cats tested (Fig. 3A). Nicotine ipc after procaine washout significantly reduced the mean iEMG activity in the right LG muscle by 45% and reduced the mean iEMG activity in the left LG muscle by 25% ($P < 0.08$) compared with ipc saline (Table 1). Similar to the effect produced during the nicotine protocol, ipc nicotine after procaine washout significantly reduced the mean iEMG activity in each LG muscle compared with both ipsilateral and contralateral TA muscles. In addition, the onset of locomotor inhibition began within two to four steps of clearing the intrapericardial cannula of nicotine. Mean iEMG activity in the TA muscles increased in response to ipc nicotine following procaine washout, but the increase was not significant compared with ipc saline.

**DISCUSSION**

This study demonstrated that chemical stimulation of cardiac receptors inhibits MLR-induced locomotion in decerebrate cats. Cardiac receptors were stimulated by injecting nicotine into the pericardial sac. This maneuver stimulates primarily epicardial receptors and evokes the well-known coronary chemoreflex. The afferent arm of this depressor reflex travels from the heart via the vagus nerves (8, 17, 18, 29, 30). Nicotine ipc significantly attenuated by 24–45% iEMG activity in hindlimb extensor muscles. The flexor muscles were not affected consistently by ipc nicotine. Procaine ipc reversibly blocked both the locomotor inhibition and the depressor reflex. It is likely that the anesthetic effect of procaine on cardiac afferent nerve activity partially lingered after the washout protocol, because the nicotine-induced decrease in blood pressure and attenuation of iEMG activity in one muscle (left LG) did not reach statistical significance ($P < 0.08$). These results are consistent with recent findings that the coronary chemoreflex has a somatic component that inhibits the knee-jerk reflex (22). These findings indicate the presence of a neural pathway from the heart...
whereby endogenous stimuli could reflexly alter motor control.

For more than two decades it has been known that afferent fibers traveling in the vagus nerves can reflexly depress somatomotor activity (9, 12, 27). In the chloralose-anesthetized cat, the knee-jerk, monosynaptic stretch, and polysynaptic muscle reflexes are depressed by intravenous injection of nicotine, phenyl diguanide, and veratradine (9, 12, 14). Comparisons between circulatory times and response latencies after injection suggest that nerve endings in the cardiopulmonary region, i.e., between the right atrium and the carotid sinus, initiate the reflex depression (12, 14). The effect is a vagal reflex, because cutting the cervical vagus nerves abolishes the depression of somatomotor activity in the cat. Unmyelinated vagal fibers from the lung clearly contribute to the depression of somatomotor activity (12). Similarly, in the chloralose-anesthetized dog, chemical and mechanical stimulation of pulmonary C fibers traveling in the vagus nerves inhibit the knee-jerk reflex (5). It is interesting that inhalation of tobacco smoke in humans also diminishes the amplitude of the knee-jerk reflex by as much as 60%, the effect being proportional to the nicotine content of the tobacco (4). The animal experiments suggest that the effect in humans can be mediated by a vagal reflex from the cardiopulmonary region.

The original findings by Ginzel and Eldred (11) and Paintal (20) that cardiopulmonary reflexes depress somatomotor activity in cats led to the proposal that this viscerosomatic reflex may safeguard against muscular overexertion. In individuals with cardiac or pulmonary insufficiency, increases in lung inflation or increases in blood pressure during exercise could stimulate cardiopulmonary afferents. Ginzel and Eldred (11) hypothesized that the activation of mechanically sensitive visceral afferents from either the heart or the lung would provide negative feedback to the somatomotor system, reflexly decreasing motor activity and thereby diminishing exercise-induced demands on the cardiopulmonary system.

Evidence from experiments in animals supports the hypothesis that mechanical and chemical stimulation of sensory nerve endings in the cardiopulmonary region can reflexly inhibit locomotion. Swimming movements are inhibited by chemical stimulation of branchial nerve fiber endings in the gills of the unanesthetized spiny dogfish (26). Locomotion performed by conscious cats and rats and by decerebrate cats is inhibited by intravenous injection of phenyl biguanide (10, 15, 24). The effect in decerebrate cats is a vagal reflex, because cutting the vagus abolishes the inhibition. Pickart et al. (24) attempted to simulate a natural mechanical stimulus that would activate receptors in the lung. They increased left atrial pressure by using a balloon placed in the left atrium of a decerebrate cat. Increased left atrial pressure inhibited MLR-induced locomotion via a vagal reflex. These findings led to the speculation that stimulation of sensory endings in the cardiopulmonary region may contribute reflexly to the exercise intolerance displayed by individuals with congestive heart failure (24).

Several investigators have attempted to determine the specific contribution of cardiac receptors to the reflex inhibition of locomotion. O’Hagan et al. (19) used a unique preparation, the conscious rabbit moving on a treadmill. In the conscious rabbit, cardiac but not pulmonary receptors reflexly evoke vagally mediated cardiovascular and respiratory responses to phenyl biguanide (2). Chemical stimulation of cardiac receptors by using intravenous phenyl biguanide does not inhibit locomotion in the conscious rabbit (19). Similarly, in the unanesthetized spiny dogfish, chemical stimulation of the heart by using phenyl diguanide does not affect the swimming behavior of the fish (26). However, the presence of a coronary chemoreflex is uncertain in this species, because changes in blood pressure and heart rate were not determined during the experiments (26). In addition, O’Hagan et al. (19)
conclude that chemical stimulation of pulmonary but not cardiac receptors is responsible for somatomotor inhibition in the conscious dog. These findings from conscious preparations using the rabbit, fish, and dog differ from the results of the present study and suggest that the effect from cardiac receptors may be species specific and/or depend on the degree to which the neuraxis is intact. The effect of cardiac receptors on the reflex inhibition of locomotor activity in conscious cats in not known. Similarly, the effect of cardiac receptors on somatomotor inhibition in humans is not known.

Nicotine ipc reduced systolic blood pressure to 88 mmHg during protocol 2 (Fig. 1B), but it is unlikely that the low blood pressure caused the locomotor inhibition. During protocol 4, ipc nicotine reduced systolic blood pressure to only 108 mmHg (Fig. 1D), yet locomotor inhibition still occurred (Fig. 3A, Table 1). The analog recording from one cat (Fig. 2B) shows that ipc nicotine attenuated iEMG activity in the left and right LG muscles at a time when the coronary chemoreflex had reduced mean arterial blood pressure to only 125 mmHg. Direct evidence from decerebrate cats demonstrates that mean blood pressure as low as 47 mmHg (systolic pressure ~57 mmHg) does not inhibit MLR-induced locomotion (24). Similarly, in the conscious rabbit, a reduction in mean blood pressure to 52 mmHg evoked by the coronary chemoreflex does not inhibit treadmill locomotion (19). The conclusion that
the low blood pressure did not cause the locomotor inhibition in the present study with the lack of an acute effect of low blood pressure on the knee-jerk reflex. Low blood pressure caused by lung inflation (5), by ipc injection of nicotine (22), or by intravenous injection of nicotine, phenyl biguanide, veratradine, or capsaicin (5, 12) does not inhibit the knee-jerk reflex. In the present experiments, if reduced blood pressure caused the inhibition, it seems reasonable to expect that iEMG activity in the TA muscles would have decreased also.

Several factors make it unlikely that extra-cardiac receptors were responsible for the inhibition of locomotion. First, the latency to the onset of inhibition was short, occurring within 5 s (Fig. 2). Cardiac afferents that respond to ipc nicotine discharge within 0.5–12 s after injection; the majority discharge in <2–5 s (18, 30). Second, nicotine did not leak from the pericardial sac. The absence of leakage was confirmed at the end of each experiment by injecting a volume of saline at least equal to the volume used during the experiment. Third, the technique of injecting ipc procaine has been used previously to block cardiovascular reflexes mediated by cardiac afferents (1, 2, 29). Procaine (1% ipc) has been shown to block the discharge of vagal afferents from the heart, but ipc procaine as concentrated as 2% does not affect the discharge of extra-cardiac vagal afferents in cats (1).

The strength of the reflex inhibition of somatomotor activity initiated by cardiac receptors may be less than that initiated by pulmonary receptors. In the present study, ipc nicotine completely abolished iEMG activity during MLR-induced locomotion in 4 of 13 cats and attenuated iEMG activity in 7 of 13 cats. In a previous study, intravenous phenyl biguanide completely abolished iEMG activity during MLR-induced locomotion in 7 of 13 cats and attenuated iEMG activity in 4 of 13 cats (24). These differences, however, may be more apparent than real. The use of different pharmacological agents may account for the apparent differences. Larger sample sizes may also reveal little difference in the reflex effects caused by stimulation of cardiac vs. pulmonary receptors. Nonetheless, although sensory receptors in the heart can reflexly inhibit locomotion in the decerebrate cat, it is possible that afferent input from the lungs and the heart combine to produce a stronger reflex inhibition of locomotion.

The present findings demonstrate that stimulation of cardiac receptors inhibits locomotion in the decerebrate cat, but the inhibition cannot be attributed to cardiac receptors responsive exclusively to either mechanical or chemical stimuli. Vagally innervated cardiac receptors responsive to epicardial nicotine can also be responsive to moderate mechanical forces applied at the epicardial surface (18). The distinction between receptor types may be important, because chemically sensitive and mechanically sensitive cardiac receptors can evoke vagal reflexes opposite in sign (13). In addition, the potential impact of an inhibitory somatomotor reflex from the heart could depend on the population of cardiac receptors activated by a physiological or pathophysiological condition. Zucker et al. (32) have shown that during heart failure, cardiovascular reflexes initiated by vagally innervated, chemically sensitive cardiac receptors are augmented, whereas cardiovascular and renal reflexes initiated by vagally innervated, mechanically sensitive cardiac receptors are diminished. The augmentation is produced, at least in part, by increased receptor sensitivity (32). Conversely, in heart failure, mechanically sensitive cardiac receptors are desensitized. The possibility exists that the combined vagal activity from pulmonary receptors and chemically sensitive cardiac receptors could contribute to impaired exercise capacity displayed by patients with congestive heart failure.

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