Role of the insular cortex in the modulation of baroreflex sensitivity

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Saleh, Tarek M., and Barry J. Connell. Role of the insular cortex in the modulation of baroreflex sensitivity. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1417–R1424, 1998.—Cervical vagal stimulation for 2 h results in a depressed baroreflex sensitivity produced by an enhanced sympathetic output, as indicated by increased plasma norepinephrine levels. The current study examined the role of the insular cortex in modulating the vagal stimulation-induced changes in baroreflex sensitivity. Male Sprague-Dawley rats were anesthetized with thiobutabarbitol sodium and instrumented for recording blood pressure, heart rate, intravenous drug administration, and vagal afferent nerve stimulation. Stereotaxic microinjections (300 nl) of either 5% lidocaine or 0.9% saline were made bilaterally into the insula. Thirty minutes after 2 h of vagal stimulation, the baroreflex was significantly depressed and plasma norepinephrine levels were significantly elevated in both groups. The baroreflex was also significantly depressed after bilateral lidocaine injections into the insula, independent of vagal stimulation. However, no significant change in plasma norepinephrine was observed, suggesting that an attenuated parasympathetic output contributed to the altered baroreflex. Taken together, the results suggest that the insular cortex modulates the cardiac baroreflex through a modulation of parasympathetic output.

SYMPATHETIC HYPERACTIVITY, as demonstrated by an elevation in plasma norepinephrine, has been strongly correlated to the depression in the baroreflex sensitivity observed immediately after the onset of several cardiovascular pathologies (1, 9, 10, 16, 19, 20, 33, 35). Specifically, in a study by Hartikainen and colleagues (15), a significant inverse correlation between baroreflex sensitivity and plasma norepinephrine was observed in hospitalized patients 10 days after their first myocardial infarction. In addition, the decreased baroreflex sensitivity and increased plasma norepinephrine levels were significantly correlated with the degree of coronary artery narrowing in these patients (15, 17).

Experimentally, myocardial infarction can be induced by the ligation of the left descending coronary artery. In a study involving dogs, this occlusion resulted in a significant elevation in both the activity of vagal afferent fibers and plasma norepinephrine levels. In contrast, baroreflex sensitivity was significantly depressed (7). The mechanism of the increased vagal afferent discharge was suggested to be due to the presence of necrotic and noncontractile segments of the myocardium, which resulted in mechanical distortion of sensory receptor endings and therefore increased the activity of cardiac vagal afferent fibers beyond normal (7). However, the depressed baroreflex sensitivity and elevated plasma norepinephrine levels observed in cardiovascular pathology cannot be explained solely by changes in the activation of myocardial or other peripheral receptors alone. Blood pressure reduction (12), changes in left ventricular function (24), and plasma levels of atrial natriuretic factor, endothelin-1, or renin activity (14) did not correlate with changes in baroreflex sensitivity. Finally, observations of structural changes in the mechanoreceptors of hypertensive patients studied in vitro and in vivo suggested that a functional central defect rather than a peripheral one was most likely a major contributor to the observed impaired baroreflex function (8). Experiments in our laboratory have shown that after vagal afferent stimulation for 2 h, a significant depression in the cardiac baroreflex was observed along with increased plasma norepinephrine levels (27). Bilateral microinjection of the reversible anesthetic lidocaine into the parabrachial nucleus before vagal stimulation completely blocked both of these changes (27). This result raised an interesting question, namely, was the sympathoexcitation as indicated by the elevated plasma norepinephrine and the decreased baroreflex sensitivity mediated solely via the parabrachial nuclei, or were forebrain cardiorespiratory nuclei, such as the insular cortex, also involved?

The insular cortex is a forebrain autonomic nucleus involved in the integration of sensory and visceral (including cardiovascular) information from peripheral receptors (29). This region of the cortex receives topographically organized, visceral afferent information contained in the vagus nerve from a variety of subcortical nuclei involved in autonomic control (5, 25, 34). In addition, the insular cortex has been shown to have efferent connectivity with these autonomic sites (5, 34), and, of particular interest, a direct reciprocal, bilateral projection between the insular cortex and the parabrachial nucleus has been demonstrated in the rat (2, 28, 31). Unilateral lesions of the insular cortex using lidocaine or after occlusion of the middle cerebral artery in the rat and cat have been shown to elicit myocardial damage and cardiac arrhythmias concomitant with an elevation in plasma norepinephrine (6, 13, 21, 32). Neurogenically mediated cardiac arrhythmias such as those observed after an initial myocardial infarction or stroke have been implicated as major contributors to sudden cardiac death (13, 21).

Therefore, the current study was undertaken to investigate the role of the insular cortex in modulating the sensitivity of the baroreflex. In addition, the role of
the insula in mediating the depressed cardiac baroreflex sensitivity and increased plasma norepinephrine levels observed after 2 h of vagal stimulation was also investigated.

MATERIALS AND METHODS

All experiments were carried out in accordance with the guidelines of the Canadian Council on Animal Care and were approved by the University of Prince Edward Island Animal Care Committee.

General surgical procedures. Experiments were performed on a total of 32 male Sprague-Dawley rats (Charles River; Montreal, PQ, Canada) weighing 250–290 g. Rats were anesthetized with thiobutabarbitol sodium (Inactin; Research Biochemicals International, Natick, MA; 50 mg/kg ip). A polyethylene catheter (PE-50; Clay Adams, Parsippany, NJ) was inserted into the right femoral artery to monitor blood pressure and heart rate and into the right femoral vein (PE-10) for the intravenous administration of drugs. Arterial blood pressure was measured with a pressure transducer (Gould P-23 ID; Cleveland, OH) connected to a Gould model 2205S polygraph. Heart rate was determined from the pulse pressure using a Gould tachograph (Biotach). An endotracheal tube was inserted, and animals were ventilated with room air to facilitate respiration.

Vagus nerve stimulation. The left vagus nerve was located through a midline cervical incision, isolated, and placed on stainless steel electrodes that were fixed in place with dental impression material (Ash Temple, Bedford, NS, Canada). The cervical vagus nerve was crushed distal to the stimulating electrodes, permitting activation of visceral afferents only. The stimulus intensity (0.5–1 mA) used to activate vagal afferents was determined using a 5-s train of pulses (50 Hz and 2 ms pulse duration; 1 s on/2 s off cycle) to produce a maximal reflex decrease in heart rate (ranging from 70 to 110 beats/min) and blood pressure (30–40 mmHg). The 1 s on/2 s off cycle of 2-h duration was chosen to prevent visceral afferent activation-induced adaptation observed with sustained visceral activation (18, 26).

Insular cortex injections. After vagal nerve isolation, the animals were subsequently placed in a David Kopf (Tujunga, CA) stereotaxic frame, and small burr holes were made bilaterally in the parietotemporal bone to allow for the stereotaxic insertion of a 30-gauge stainless steel, 1-µl Hamilton microsyringe into the insular cortex according to coordinates obtained from a stereotaxic atlas (23). To reversibly interrupt neurotransmission, bilateral microinjections of the anesthetic lidocaine (5%, 300 nl per side; Sigma, St. Louis, MO). The changes in blood pressure and heart rate in response to activation of the cardiac baroreflex using PE (0.025, 0.05, and 0.1 mg/kg) was monitored 30 min before and

30 and 120 min after termination of the vagal stimulation protocol.

The peak amplitude changes in mean arterial pressure and mean heart rate evoked by each concentration of PE were plotted against each other to provide an index of baroreflex sensitivity. Regression lines were obtained by least squares method, and the slopes of the lines before and after vagal stimulation were calculated. This plot of baroreflex sensitivity was used to analyze changes in the slope (sensitivity) of the baroreflex function curves before and after vagal stimulation and with or without microinjections of lidocaine into the insula.

Plasma catecholamine measurements. Because baroreflex testing using PE may interfere with plasma catecholamine measurements, additional rats were instrumented as described in Insular cortex injections and used to measure plasma catecholamine changes but were not used to test the baroreflex [8 stimulated (4 lidocaine, 4 saline) and 8 nonstimulated (4 lidocaine, 4 saline)]. Approximately 1 h after determination of the maximal reflex stimulation, blood samples (500 µl) were taken every hour for 5 h, and, in addition, a blood sample was taken 30 min after termination of the vagal stimulation. Blood was allowed to flow freely into an Epidendorf tube directly from the indwelling arterial catheter and was spun in a microcentrifuge (14,000 revolutions/min) for 1 min. The plasma was removed with a pipette and transferred to heparinized blood collection tubes (Amersham Life Sciences, Oakville, ON, Canada) that were stored at −20°C before catecholamine extraction and analysis. Unconjugated norepinephrine and epinephrine were measured in duplicate using a modification of the Biotrak (Amersham Life Sciences, Oakville, ON, Canada) radioenzymatic assay as previously described (22). Assay sensitivities, calculated as the amount of catecholamine needed to produce a sample cpm:blank cpm ratio of 2, were 6 pg for norepinephrine and 6 pg for epinephrine.

Data analysis. All data are presented as means ± SE of the mean and were analyzed by a one-way ANOVA for repeated measures followed by a Student-Newman-Keuls post hoc analysis. Analysis of covariance (ANCOVA) was used when comparing differences between multiple regression lines. Differences were considered significant if P < 0.05.

RESULTS

In all animals receiving PE injections (0.025, 0.05, and 0.1 mg/kg), before any treatments (n = 16), the average changes in blood pressure, heart rate, and the slope of the baroreflex sensitivity plot (average slope = 0.49 ± 0.03; Fig. 1A) were not significantly different from each other (P > 0.05). In addition, baseline levels of plasma norepinephrine and epinephrine were not significantly different between groups (n = 16, P > 0.05). Vagal stimulation resulted in an average decrease in heart rate (30 ± 11 beats/min) and mean arterial pressure (42 ± 12 mm Hg) compared with prestimulated values for all stimulated animals used in this study. Further, termination of the vagal stimulation after 2 h resulted in an average increase in heart rate (22 ± 18 beats/min) and mean arterial pressure (38 ± 22 mm Hg) with respect to the corresponding stimulated values. These cardiovascular parameters were not significantly different from prestimulated values (P > 0.05).
Insular cortex microinjections and vagal stimulation. Intravenous injection of PE (0.1 mg/kg) before vagal stimulation resulted in an average increase in mean arterial pressure (37 ± 8 mmHg) and reflex decrease in heart rate (18 ± 5 beats/min; Fig. 1A). Microinjection of saline (0.9%, 300 nl/side) into the insular cortex did not result in significant changes in either mean arterial pressure or heart rate (P > 0.05) compared with the preinjection baseline values (Fig. 1A). When the baroreflex was evoked 30 min after the 2 h of vagal stimulation, a significantly enhanced pressor response (61 ± 8 mmHg; P < 0.05) independent of a significant change in the reflex bradycardia (14 ± 6 beats/min, P > 0.05), was observed (Fig. 1; A, B, and B2). This enhanced pressor response was observed only at the highest concentration of PE used (0.1 mg/kg, Fig. 1B1).

When the average changes in mean arterial pressure were plotted against the average changes in heart rate in response to various concentrations of PE, the slope of the regression line obtained 30 min after termination of the vagal stimulation was significantly decreased (0.32 ± 0.06, P < 0.05) compared with the slope of the regression line before stimulation (0.48 ± 0.01; Fig. 1, C1 and C2). The slope of the baroreflex sensitivity returned to approximate prestimulated values after an additional 90 min (0.485 ± 0.01, P < 0.05; Fig. 1, C1 and C2).

Microinjection of lidocaine (5%, 300 nl/side) into the insular cortex did not result in significant changes in either mean arterial pressure or heart rate (P > 0.05) compared with the microinjection of saline into the cortex (Fig. 2A). In animals receiving lidocaine microinjections before stimulation, baroreflex testing 30 min after termination of the stimulation resulted in a significantly enhanced pressor response (75 ± 7 mmHg, 49).
Intravenous injection of PE (0.1 mg/kg) resulted in a significant increase in mean arterial pressure (MAP) and a decrease in heart rate (HR), as shown in Fig. 2A. This response was observed in all animals tested. The average changes in MAP and HR were plotted against the concentrations of PE used (Fig. 2B). The slope of the regression line obtained 30 min after termination of vagus nerve stimulation was significantly decreased (0.23 ± 0.04, P < 0.05) compared to the slope before stimulation (0.47 ± 0.01; Fig. 2C). The slope of the baroreflex sensitivity returned to approximate prestimulated values after an additional 90 min (0.48 ± 0.01, P > 0.05; Fig. 2D).

Insular cortex microinjections independent of vagal stimulation. When the baroreflex was tested 30 min after the bilateral microinjection of lidocaine into the insular cortex, a significantly attenuated reflex bradycardia (7 ± 3 beats/min compared with 17 ± 3 beats/min before lidocaine, P < 0.05; Fig. 3A) was observed. This attenuated bradycardic response to an increase in blood pressure was observed at all concentrations of PE used (Fig. 3B). As a result, the slope of the baroreflex sensitivity was significantly decreased to 0.23 ± 0.02 (P < 0.05) compared to a slope of 0.48 ± 0.01 before the microinjection of lidocaine into the insular cortex (Fig. 3C).

Bilateral saline microinjections (n = 4) into the insula did not result in significant changes in baseline cardiovascular values (P > 0.05), or the slope of the baroreflex sensitivity (P > 0.05), in nonstimulated animals compared with preinjection values (results not shown).

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**Fig. 2.** A: arterial blood pressure (mmHg) and HR (beats/min) responses to bolus intravenous injection of PE (0.1 mg/kg) as indicated by arrows. Cardiovascular responses to PE administration were taken from a continuous record from an experimental animal 30 min before and during bilateral microinjection of lidocaine into IC. Time of injection is represented by horizontal line above trace. Also shown are cardiovascular responses 30 and 120 min after termination of vagus nerve stimulation (2 h stim). Time scale is shown at bottom right. B: peak changes in MAP (B1) and HR (B2) to increasing concentrations of PE 30 min before and 30 and 120 min after termination of vagus nerve stimulation. *Significant difference (P < 0.05) from prestimulated values. C1: plot of baroreflex sensitivity. Average change in MAP vs. average change in HR to intravenous injection of increasing concentrations of PE 30 min before and 30 and 120 min after 2 h of vagus nerve stimulation in animals receiving lidocaine microinjections into IC. Vertical and horizontal lines represent SE. Regression lines were calculated by least squares method, and all had an r² value > 0.9. C2: histogram showing slopes for each regression line (n = 4 for each group). *Significant difference (ANCOVA; P < 0.05) from prestimulated (before) slope.
Lidocaine microinjections into the insular cortex and changes in plasma catecholamines. Blood samples were assayed for plasma catecholamines, and the values before, during, and after vagal stimulation with either lidocaine or saline microinjections were compared (Fig. 4). Similar to previous results observed by these investigators (27), a significant decrease (to 195 ± 687 pg/ml) followed by a significant increase (to 1,390 ± 179 pg/ml) in plasma norepinephrine levels was observed during and after vagal stimulation, respectively, after the microinjection of saline into the cortex (Fig. 4A). These changes in plasma norepinephrine were observed independent of any significant changes in plasma epinephrine levels throughout the experimental time course.

When the above protocol was repeated with the addition of lidocaine into the insular cortex, both the decrease in plasma norepinephrine during (to 375 ± 67 pg/ml) and increase immediately after (to 1,400 ± 187 pg/ml) vagal stimulation were not significantly different from those observed after saline microinjections into the insular cortex (Fig. 4A, P > 0.05).

Again, plasma epinephrine levels remained relatively unchanged throughout the experimental time course (Fig. 4B, P > 0.05). Also, in all nonstimulated animals receiving either saline or lidocaine microinjections into the insular cortex, no significant changes in either plasma epinephrine or norepinephrine levels were measured throughout the experimental time course (Fig. 5, A and B; P > 0.05).

Histological verification of cannula placement. Figure 6 shows a composite diagram indicating the tips of the microinjection cannulas in the region of the insular cortex (agranular, granular, and dysgranular) obtained from all animals used in this investigation. Data from animals whose lesions were located outside the insula
were not included in this study. The results from such animals did not demonstrate any significant changes in the slope of the baroreflex sensitivity (data not shown).

**DISCUSSION**

Our results demonstrate that blockade of synaptic transmission through the insular cortex significantly decreases the slope of the baroreflex sensitivity. This was evident based on the finding that blockade of the insula with local lidocaine microinjection independent of vagal stimulation resulted in a significantly attenuated reflex bradycardic response to an evoked increase in blood pressure. The attenuated bradycardia observed after blockade of the insula is likely the result of a lidocaine-induced inhibition of parasympathetic tone. A decrease in parasympathetic tone would reduce the magnitude of the reflex bradycardia, resulting in a reduction in the slope of the regression line for baroreflex function. An inhibition of parasympathetic tone is further suggested by the lack of a significant change in plasma norepinephrine levels, indicating no significant change in sympathetic tone. A similar reduction in the slope of the regression line for baroreflex function was observed after microinjections of either saline or lidocaine into the insula before 2 h of vagal stimulation. However, in this case, an enhanced pressor response to PE injection occurred with only a slight enhancement of the reflex bradycardia. This enhanced pressor response was likely due to an increase in sympathetic tone, as indicated by the elevation in circulating plasma catecholamines.
norepinephrine. Changes in parasympathetic tone were not likely involved in mediating this effect because there was no significant attenuation of the reflex bradycardia.

Under normal circumstances, it seems that the role of the insular cortex is to increase parasympathetic tone. When a bilateral lesion of the insular cortex is combined with vagal stimulation for 2 h, a depression in the baroreflex sensitivity is observed. However, the mechanism involved after vagal stimulation seems to involve an enhanced sympathetic tone, as demonstrated by the enhanced pressor response to PE injections and increased norepinephrine levels 30 min after termination of the vagal stimulation.

The precise role of the insular cortex in cardiovascular regulation is not clearly understood. It appears that due to the topography of visceral inputs to this region, as well as the differences in the responses obtained after lesions of the right versus left insular cortices, the role of the insula in autonomic regulation remains difficult to interpret. For example, in our study, bilateral lidocaine microinjections into the insular cortex did not significantly change baseline blood pressure, heart rate, or plasma norepinephrine. This would seem to indicate that the insula has no tonic input onto sympathetic or parasympathetic preganglionic nuclei.

Further, Butcher and Cechetto (3) demonstrated that a unilateral microinjection of lidocaine into the left insular cortex did not affect either baseline cardiovascular variables or sympathetic nerve activity. Similarly, when a lesion of the left insula was done by occluding the middle cerebral artery, no changes in either baseline cardiovascular variables or plasma norepinephrine levels were observed (4). Hachinski and colleagues (13) utilized the same middle cerebral artery occlusion protocol to also demonstrate that lesions of the left insula do not result in significant changes in baseline cardiovascular parameters. However, lesion of the right insula resulted in an increase in sympathetic nerve activity with no accompanying changes in baseline blood pressure or heart rate (13). Finally, consistent with the results presented in the current study, occlusion of the common carotid artery, which effectively results in a bilateral lesion of both insular cortices, resulted in no observable change in either plasma catecholamine levels or cardiovascular parameters (6).

Although the above mentioned studies suggest that the insula does not play a role in tonic autonomic control, the results of this study are the first to suggest that the insula may be involved in modulating baroreflex sensitivity. Our results suggest that bilateral blockade of the insular cortex results in an attenuated reflex bradycardia and decreased slope of the baroreflex sensitivity likely due to parasympathetic withdrawal. Therefore, this result clearly indicates an involvement of this nucleus in modulating baroreflex sensitivity. This cortical role is somewhat obscured after vagal stimulation, because the slope of the baroreflex is not further affected by cortical blockade. However, because the decrease in the reflex bradycardia associated with insular lesions (Fig. 3B2) is no longer present after vagal stimulation (Fig. 2B2), we can conclude that the vagal stimulation may in fact be inhibiting parasympathomimetic neurons in the insula.

Perspectives

Substantial evidence is provided in the literature to suggest that the existence of a strong inverse correlation between plasma norepinephrine and baroreflex sensitivity is a possible mechanism for the lethal cardiac arrhythmias associated with sudden death (14). This has been observed in several cardiovascular pathologies, including congestive heart failure (11, 12), experimentally induced myocardial infarction (30), and hypertension (16). We suspect that the significant depression in baroreflex sensitivity observed after blockade of the insular cortex in the present study may be an important mechanism responsible for the cardiac effects observed after a stroke involving this region. Because no change in plasma norepinephrine was observed after lesion of the insula, the mechanism resulting in a depressed baroreflex sensitivity appears to be due to a change in parasympathetic tone. Although this is the only study in which baroreflex sensitivity has been measured after lesion of the insular cortex, it is tempting to suggest that the depression in baroreflex sensitivity is an indication of underlying cardiac electrophysiologic changes and possible damage to the myocardium eventually leading to lethal cardiac arrhythmias and sudden death.

The authors acknowledge Monique C. Saleh for helpful comments in preparing and reviewing this manuscript.
This investigation was supported by an internal Atlantic Veterinary College Grant (no. 612128) awarded to T. M. Saleh.

Received 7 October 1997; accepted in final form 28 January 1998.

REFERENCES


