THE EFFECT OF ACTIVATION OF CAROTID BODY CHEMORECEPTORS ON BARORECEPTOR INHIBITION OF SYMPATHEtic ACTIVITY

L. CHRUSCIELEWSKI, S. MAJCHERCZYK and A. TRZEBSKI

Department of Physiology, Institute of Physiological Sciences Medical Academy Krakowskie Przedmieście 26/28, 00-927 Warsaw, Poland

Key words: sympathetic activity, baroreceptor, chemoreceptor

Abstract. The effect of combined stimulation of peripheral chemo- and baroreceptors upon sympathetic discharge was studied in cats anesthetized with chloralose and urethan, immobilized with tricuran, and artificially ventilated. The right carotid sinus was arterially isolated, the left sinus nerve and both vago-sympathetic trunks were cut. A rise of pressure in isolated carotid sinus from 100 to 200 mm Hg produced an inhibition of sympathetic activity recorded simultaneously from the inferior cardiac and the renal nerves. For a combined chemo- and baroreceptor stimulation, the carotid sinus was perfused at a constant pressure of 100 mm Hg with venous blood bubbled with CO₂. Twenty seconds after the onset of the perfusion the carotid sinus pressure was raised up to 200 mm Hg. A combination of the chemoreceptor stimulation and of the rise of sinus pressure produced only a small inhibition of sympathetic activity in both nerves compared to the effect observed without concomitant chemoreceptor activation. It was demonstrated that chemically induced stimulation of the carotid body chemoreceptors strongly influences the magnitude of the sympathetic inhibition produced by activation of the baroreceptor reflex.
INTRODUCTION

Activity of the sympathetic nervous system is modulated by afferent inputs from baroreceptors as well as from peripheral chemoreceptors. The carotid sinus reflex which evokes reduction of systemic blood pressure following distention on the carotid sinus is thought to be due to a decrease of sympathetic activity. Excitation of peripheral chemoreceptors evokes sympathetic discharge, producing an increase of blood pressure. Since both the chemo- and baroreceptors play a part in central nervous integration of some cardiovascular functions, it is important to clarify how the circulation is affected by the simultaneous activation of these peripheral receptors. Reflex vascular responses to simultaneous chemo- and baroreceptor activation were recently studied (6), but the knowledge of the modulation of sympathetic outflows by a combined activation of these receptors is still unsatisfactory.

The purpose of this study was to examine the response of combined chemo- and baroreceptor stimulation on sympathetic activity. In 1963 Downing and Siegel (3) showed that stimulation of carotid chemoreceptor with hypoxic blood had practically no effect on sympathetic discharge recorded from the inferior cardiac nerve. On the other hand, Trzebski et al. (13, 14) recently reported an increase in activity of the inferior cardiac nerve to stimulation of the carotid chemoreceptors. In this study we recorded activity from renal and inferior cardiac sympathetic nerves assuming that if there is any specificity in their responses, the pattern of response to combined chemo- and baroreceptor stimulation should be different in these two nerves. The work described here has been published under the form of an abstract (2).

METHODS

Twenty five cats weighing between 2.5 and 5.2 kg were anesthetized with ethyl chloride and then given chloralose — (40 mg/kg) and urethane (200 mg/kg) via a forelimb vein. A catheter was introduced into the left femoral artery and the arterial blood pressure recorded with the use of a pressure transducer (Statham 23 AC). Mean pressures were obtained electronically. This catheter was also used for anaerobic withdrawal of arterial blood samples, which were analyzed in vitro for pH, pCO₂ and pO₂. Blood gases were measured by the micro-Astrup System. The cats were paralyzed with gallamine triethiodide (Tricuran) 5 mg/kg and artificially ventilated. Supplemental doses (2 mg/kg i.v.) of Tricuran were administered, as required, during the course of the experiment.
To maintain constant ventilation, pneumothorax was made by opening a hole in the thoracic cavity. The respirator rate was adjusted to maintain arterial blood $pCO_2$, $pO_2$ and $pH$ in the normal range. Sodium bicarbonate was administered when necessary to maintain blood $pH$ between 7.3 and 7.4. To expose the carotid body and sinus nerve, the trachea and oesophagus were transected in the neck and reflected cranially in the midline. Both vago-sympathetic trunks were isolated and sectioned. The cats were heated so as to maintain their rectal temperature at 37–38°C.

Fig. 1. Diagrammatic representations of the experimental procedures used for perfusion of the carotid sinus. CC, common carotid artery with loop inserted; SCG, superior cervical ganglion; AP, ascending pharyngeal artery; XII, hypoglossal nerve; EC, external carotid artery; LA, lingual artery with cannula and stopcock; SN, sinus nerve. Modified figure from Purves (10).

The method of perfusing the carotid body with arterial blood at known pressures was similar to that described by Purves (Fig. 1). The cat was given heparin (15 mg/kg intravenously) and a 20 cm loop with a side arm of polyethylene tubing was inserted into the common carotid artery. The dead space of the side arm and the cephalic half of the loop was approximately 1 ml. All the branches of the common and external carotid arteries between the loop and the lingual artery catheter were tied as far distal from the carotid body as possible to avoid any effects of retrograde clotting. Special care was taken to leave the carotid body venous drainage intact.

The lingual artery was cannulated retrogradely so that the cannula tip lay approximately 5 mm above the carotid sinus and pressure within the carotid sinus was measured continuously with a Statham (23 AC) transducer. The external carotid artery could be closed intermittently by tightening a linen thread snare around the lingual catheter. The carotid sinus could be isolated by clamping the cardiac side of the loop and any desired pressure could be applied within the arterial segment by means of a sphygmomanometer attached to the stopcock on the
sidearm. When a recording at constant pressure was to be made, the sidearm of the loop was filled with carotid artery blood, the carotid sinus segment was isolated by snare and clamps, and a pressure of 100 mm Hg was applied. In order to stimulate baroreceptors the carotid sinus pressure was then increased rapidly from 100 mm Hg value and maintained at an increased level for 15–30 s. The effects of two procedures on sympathetic activity were compared. The first procedure consisted of raising the pulse pressure without changing the tension of blood gases. In the second procedure, besides raising the pressure, chemoreceptors were concomitantly stimulated. Chemoreceptor stimulation was achieved by filling the loop via the lingual artery catheter with chemoreceptor stimulating agents. The time of perfusion period did not exceed 90 s, and tests did not start until serial blood gas and pH measurements indicated that the animal was in steady state condition. At the end of each test involving chemoreceptor stimulation, the stimulating agents were sucked out from the loop through the lingual artery catheter. Between recordings a normal systemic blood flow through the carotid sinus was reinstated for 10 min. The contralateral sinus nerve was identified anatomically and by recording its pulsatile nerve activity and subsequently transected. Since it was possible that the ipsilateral sinus nerve could have been damaged during isolation, the integrity of afferent carotid body chemoreceptor and baroreceptor afferent pathway was tested in each experiment after initial dissection and again towards the end of the experiment. The tests consisted of (i) rising pressure within the carotid sinus by 100 mm Hg above systemic arterial pressure and observing a fall of femoral arterial pressure, and (ii) perfusing normal saline bubbled with 100% CO₂ into the common artery loop at constant pressure, the response consisting of a rise in systemic pressure. All 26 cats were tested to graded rises in carotid sinus pressure, but only in 3 cats out of 26 sympathetic activity responded to the relatively small rises in sinus pressure. In the remaining cats only strong stimulation of carotid pressoreceptors (100–200 mm Hg) produced an inhibition of sympathetic activity, presumably due to depressing effect of chloralose anesthesia on baroreflex. Since a rise of carotid sinus pressure of this magnitude is known to inhibit chemoreceptor discharge, such chemoreceptor stimulation agents had to be chosen which produced strong and continuous activation of carotid chemoreceptors, independent of very strong rises of carotid sinus pressure.

In three cats chemoreceptor activity was analyzed while infusing different agents into the carotid loop. Increase in chemoreceptor activity induced by infusion of arterial or venous blood equilibrated with 100% CO₂ was found not to be affected by marked rises in carotid sinus
pressure (100–200 mm Hg). In three cats carotid sinus was arterially isolated and perfused with pulsatile pressure. Recordings were made from single afferent baroreceptor fibers during the infusion on chemoreceptor stimulating agents used in this study. Perfusion of carotid sinus area with venous or arterial blood equillibrated with 100%/CO₂ had no effect upon the discharge. Renal, and cardiac sympathetic nerves were dissected and cut peripherally. With the aid of a dissecting microscope the sympathetic nerves were freed from their connective tissue just before recording and placed in a warm paraffin pool. The nerve impulses were detected by bipolar electrodes consisting of a pair of platinum wires spaced 3–5 mm apart. The action potentials were amplified using the Gallileo type R 36 zm preamplifier. Usually, the activity from the two sympathetic postganglionic nerves was simultaneously displayed on an oscilloscope and recorded on polygraph (Gallileo). The stimulation of carotid sinus baroreceptors caused reflex vasodepression and immediate inhibition of efferent sympathetic activity. Since the duration of inhibition of sympathetic outflow depends on the strength of stimulus applied to baroreceptors, we decided to use the duration of inhibition of sympathetic outflow to a standard rise in carotid sinus pressure (from 100 to 200 mm Hg) as an index of sensitivity of the baroreflex. Duration of inhibition in control recording was computed and compared to the duration observed when combined chemo- and baroreceptor stimulation was applied. The significance of the changes was determined by Student's t-test for paired observation.

RESULTS

Effects of stimulating baroreceptors only. The most obvious feature of the response was an immediate cessation of renal and cardiac sympathetic activity. Some adaptation to carotid sinus distension was observed; its inhibitory effect on sympathetic discharge was maximal at the beginning but gradual diminished after 3–6 s of sustained pressure.

In three cats sympathetic activity was specially sensitive to baroreceptor stimulation and even a slight elevation of the carotid sinus pressure (from 100 to 120 mm Hg) was able to produce an inhibition of sympathetic discharge. The inhibition of the sympathetic activity to the baroreflex progressively increased when the baroreceptors were stimulated by further gradual elevation of carotid sinus pressure (from 100 to 140 and from 100 to 160 mm Hg). Systemic arterial pressure began to fall about 2–5 s following the distention of the carotid sinus and remained reduced throughout the period of carotid sinus baroreceptor stimulation in all experiments.
A graded excitation of the baroreceptors during a constant chemo-
receptor stimulation. In three cats in which sympathetic discharge was
especially sensitive to baroreceptor stimulation, the interaction
between chemo- and baroreceptor was tested at different levels of
activation of those receptors.

Control, graded stimulation of baroreceptors was performed by in-
creasing the pressure of the arterial blood perfusing the sinus in steps,
starting each time from 100 mm Hg: 100–120, 100–140, 100–160 mm Hg.
The identical procedure was repeated again during strong (venous
blood + 100%/ CO₂) and weak, venous blood pH 7.22 (7.11–7.34); pCO₂
48 mm Hg (34–62); pO₂ 40.5 mm Hg (30–51), chemoreceptor stimulation.
Figure 2 illustrates a typical response observed in all three cats to
graded baroreceptor stimulation when an arterial blood within the loop
was substituted by hypercapnic venous blood. The inhibition of sym-
pathetic discharge to all three levels of baroreceptor stimulation was
markedly reduced during strong chemoreceptor stimulation. It is of
interest that this chemo-reflex-induced reduction of inhibition was
still dependent on the strength of baroreceptor excitation, the stronger
stimulus was applied to baroreceptors the longer was the inhibition of
sympathetic discharge.

In the same three cats we tested graded baroreceptor excitation
during weaker chemoreceptor stimulation. Following a control testing
of the baroreflex, the arterial blood within the loop was replaced by
freshly collected venous blood. In those experiments a weak activation
of the chemoreceptors also reduced the period of inhibition compared
to control recording, but only when the stimulus to the baroreceptors
was relatively weak (increase of sinus pressure form 100 to 120 mm Hg).
When the carotid sinus pressure was further gradually increased (100–
140, 100–160 mm Hg), the duration of the inhibition did not differ from
that observed during exclusive baroreceptor stimulation in control re-
cordings.

Effect of strong stimuli applied simultaneously to baro- and chemo-
receptor on sympathetic activity. The effects of strong simultaneous
stimulation of chemo- and baroreceptors was studied in 23 cats. Exci-
tation of carotid body chemoreceptors following infusion of venous blood
equillibrated with 100%/ CO₂ always produced an increase in discharges
in the renal and the cardiac nerves and evoked a marked elevation of
arterial pressure (Fig. 3). In these tests the baroreceptors were stimu-
lated during the period of excitation of the carotid body chemoreceptors,
the increase of carotid sinus pressure being started about 20 s after
commencement of the infusion of the hypercapnic blood into carotid
body. When carotid sinus pressure was raised from 100 to 200 mm Hg
in order to obtain simultaneous baroreceptor stimulation, little inhibition of sympathetic activity occurred. Typical results of one such experiment are illustrated by Fig. 4.

A comparison of all the control tests of stimulation of baroreceptors
Fig. 2. Effect of graded stimulation of baroreceptors from 120 to 160 mm Hg during strong excitation of peripheral chemoreceptors with venous blood equilibrated with 100% CO₂. Symbols: A, stimulation of baroreceptors alone; B, activation of baroreceptors during strong excitation of peripheral chemoreceptors; BP, blood pressure; sbp, sinus pressure; ICN, inferior cardiac nerve.

Fig. 3. Effect of excitation of the carotid body chemoreceptors upon sympathetic activities. Traces from above downwards: electroneurogram of sympathetic activity in the inferior cardiac nerve (ICN), electroneurogram of sympathetic activity in renal nerve (RN) mean blood pressure (mbp). Arrow indicates beginning of infusion of venous blood equilibrated with 100% CO₂ into the loop. Note the increase of activity in both nerves accompanied by increase in blood pressure.

only with those performed during excitation of carotid body chemoreceptors shows that the duration of inhibition of renal and cardiac nerve activity in the latter was shorter by $6.9\pm0.86$ s, $P < 0.001$. A smaller decrease in arterial blood pressure was also observed during combined chemoreceptor stimulation. At the end of the period of distention of the carotid sinus, arterial pressure returned to the original (elevated) level as long as chemoreceptor stimulation continued.
Fig. 4. Effect of combined chemo- and baroreceptor stimulation upon sympathetic activity. Traces from above downwards: electroneurogram of sympathetic activity recorded in the inferior cardiac nerve (ICN); electroneurogram of sympathetic activity in the renal nerve (RN); mean blood pressure (MBP); pressure in the left sinus (SBP). A, control response to baroreceptor stimulation; B, carotid sinus was perfused with venous blood equilibrated with 100% CO₂ at 100 mm Hg — to obtain combined stimulation of chemo- and baroreceptors carotid sinus pressure was raised to 200 mm Hg. Note: duration of inhibition of sympathetic activities in both nerves during combined stimulation of chemo- and baroreceptors was much shorter than the one observed during exclusive stimulation of the baroreceptors.

DISCUSSION

Trzebski et al. (13, 14) have reported recently that strong stimulation of the carotid body chemoreceptors produces increased activity in the interior cardiac nerve. This study shows that a strong stimulation of the carotid body chemoreceptors produced an increase of activity in
the inferior cardiac nerve, which supports the studies of Szulczyk and Trzebski (13) and disagrees with earlier results of Downing and Siegel (3). The results of this study indicate also that chemically induced prolonged stimulation of the carotid body chemoreceptors has a very strong limiting effect on the duration and the magnitude of the carotid baroreceptor sympa-tho-inhibitory reflex. This inhibitory effect of baroreceptor activation on sympathetic activity in cardiac and renal nerves is markedly reduced, but not abolished, by a preceding strong chemoreceptor stimulation. A similar conclusion could be drawn from earlier experiments of Green and Heffron (4, 5), who also observed an increase of sympathetic activity (cardiac and vertebral nerves) during generalized hypoxia, despite a concomitant rise in blood pressure, in animals where baroreceptors were left operative. In these experiments however, central activation of the adrenergic system by hypoxia must be also taken into account (1). Stimulation of carotid chemoreceptors with hypoxic-hypercapnic blood increased renal vascular resistance by 56% in preparation where aortic baroreceptors were left intact (10). Following vagotomy the same chemoreceptor stimulation increased the resistance by 69%. Wennergren et al. (16) observed that in the presence of a continuous chemoreceptor stimulation even an intense activation of the baroreceptors was unable to lower the blood pressure to the same degree as in the case when the chemoreceptors were not excited. This however contrasted e.g., with renal vessel responses where a strong baroreceptor inhibitory stimulation completely overcame a chemoreceptor excitatory influence. In the study of Wennergren et al. (16) the responses of the renal vessels to combined chemo- and baroreceptor stimulation differ from the observations reported in this study. It should be remembered, however, that the magnitude of cardiovascular responses evoked by changes in pO₂, pCO₂ and pH at the carotid body is related to the intensity of the stimulus applied (11). Circulatory responses to baroreceptor activation, on the other hand, are also proportional to the strength of the stimulus. Different strength of the stimulus applied to carotid chemoreceptors in our experiments and in the experiments of Wennergren et al. (16) may partly explain the discrepancy between the result. In a series of experiments we compared the response in sympathetic activity to graded baroreceptor stimulation during constant chemoreceptor excitation. During strong and continuous chemoreceptor activation by venous blood bubbled with CO₂, the inhibition of the sympathetic response to the baroreflex progressively increased when the baroreceptors were stimulated by gradual elevation of carotid sinus pressure. It is of interest that less intensive continuous chemoreceptor stimulation (carotid sinus perfused with venous blood) in some of our experiments still limited the inhibition of sympathetic activity caused by baroreceptor stimulat-
ion, but only when the elevation of carotid sinus pressure was relatively small (from 100 to 1200 mm Hg). Stronger distention of carotid sinus (from 100 to 140 mm Hg) produced an inhibition of cardiac nerve activity similar to that observed when the carotid sinus was perfused with normal arterial blood.

The above-mentioned results indicate that the strength of stimulus applied to baroreceptors in relation to the intensity of chemoreceptor activation could be of great importance in determining the pattern of response to combined stimulation of these receptors. In experiments presented in this study or in those of Green and Heffron (5) and Parker et al. (10), strong and continuous stimulation of the chemoreceptors was sufficiently powerful to suppress the inhibition of the vasomotor neurons by baroreceptors. However, if the stimulus to the chemoreceptors was less intense or the stimulus applied to baroreceptors was stronger, the baroreflex gradually overcame the chemoreflex-induced excitation of the adrenergic system. This idea is also indirectly supported by results of Iriki et al. (7). Mild hypoxia (8% O\textsubscript{2} in N\textsubscript{2}) in their experiments produced an inhibition of cardiac nerve activity in rabbits where baroreceptors were left operative. This decrease was probably due to a concomitant rise in blood pressure and subsequent activation of baroreceptors as discussed by Trzebski et al. (14). When in the experiments of Iriki et al. (7) a mild hypoxia was replaced by a more severe one (3% O\textsubscript{2} in N\textsubscript{2}), cardiac nerves responded with an increase in discharge despite the marked increase in blood pressure.

The duration of chemoreceptor stimulation seems also to play an important role in determining the response to interaction between chemoreceptor and baroreceptor stimulation on sympathetic outflow.

In the experiments of Green and Heffron (4, 5), Parker et al. (10) and in our experiments, the stimulus to chemoreceptors was both strong and long-lasting in preparations where the baroreceptors were left operative. It is possible that the baroreflex in those experiments was either unable to overcome the continuous adrenergic excitation (4, 5) or the inhibition evoked by the baroreflex was relatively small (10) judging from the results obtained after denervation of the aortic baroreceptors. However, if the stimulus to the chemoreceptors was either relatively weak (16) or short lasting (8, 13, 14), baroreceptor excitation dominated the response.

The reduction of the baroreflex-induced inhibition of sympathetic outflow during combined chemoreceptor and baroreceptor stimulation in our study, may not necessarily be due to a simple addition of the excitatory and the inhibitory influence upon sympathetic discharge. Our previous observation (2) showed that an increase in sympathetic activity during central hypercapnia did not reduce the duration of the baroreflex —
evoked inhibition of the sympathetic discharge, on the contrary, the duration was even markedly extended.

Heistad et al. (6) studied the effect of interaction between baro- and chemoreceptor stimulation upon the level of arterial B. P. and gracillis muscle perfusion pressure concluding that activation of baroreceptor on one side attenuated the vasoconstrictor response to chemoreceptor stimulation on the other side. Similar conclusions are drawn from experiments of Mancia (9). The discrepancy between the results presented in this paper and the conclusions of Heistad et al. (6) and Mancia (9) could be partly explained by an entirely different sequence of stimulations of baro- and chemoreceptors in these studies. In the experiments of Heistad et al. (6) and Mancia (9), baroreceptors were continuously stimulated for some time before the chemoreceptors were activated. This contrasted with the method used in our experiments, where chemoreceptors were continuously and strongly stimulated before the baroreceptors were activated. It should be also emphasized that it is rather difficult to compare the results obtained from recordings of sympathetic activity with those in which cardiovascular parameters were analyzed. It is a well known fact that activation of carotid baroreceptors evokes a fall of blood pressure which remains reduced throughout the period of carotid sinus baroreceptors stimulation. This is not the case with sympathetic discharges. Although sympathetic outflow is markedly inhibited immediately following stimulation of the carotid sinus baroreceptors, it returns toward pre-stimulation levels at the time when the baroreceptors are still being stimulated (15). Therefore the pattern of response to combined stimulation of baro- and chemoreceptors on sympathetic outflow may not necessarily be identical to that when other cardiovascular parameters are recorded. Due to the very quick reappearance of sympathetic activity, occurring during continuous distention of the carotid sinus, it was impossible in our studies to change the sequence of stimulation. Activation of chemoreceptors could have been evoked during a period of time when sympathetic activity already started to reappear (despite continuous baroreceptor stimulation), making the drawing of any conclusions difficult.

It is of interest that the pattern of response to combined baro- and chemoreceptor stimulation differs in particular vascular beds when cardiovascular parameters are recorded (16), which contrasts with apparently uniform pattern of response in cardiac and renal sympathetic nerves from which recordings were made in our study.

We are grateful to Mr. W. Majewski for technical assistance during this work. This investigation was supported by Project 10.4.2.01 of the Polish Academy of Sciences.
REFERENCES

1. BOWER, E. A. 1975. The influence of hypercapnia and hypoxia on the activity recorded from intestinal nerves in the rabbit with cut sinus nerves. J. Physiol. 249: 68-69P.

Accepted 5 October 1978