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Phrenic nerve activity during artificial ventilation at different body temperatures and its relationships with carotid chemosensory activity

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While the chemoreceptor discharges of carotid bodies in vitro are highly dependent on temperature, these chemoreceptors in situ contribute only moderately to the ventilatory adjustment to changing body temperature (T_b), probably because of the concomitant and reverse changes in natural chemoreceptor stimuli in closed-loop preparations. Accordingly, we studied the frequency of carotid chemosensory discharge (f_x) and the phrenic integrated electroneurogram ($IENG_{ph}$) in pentobarbitone anesthetized cats, paralyzed with alcuronium and artificially ventilated, at three steady-state levels of T_b (35.5, 37.5 and 40.2° C), modifying the frequency and volume of the ventilator to maintain $P_{ET}CO_2$ within normal range. While fx increases along with T_b when $P_{ET}CO_2$ is allowed to fluctuate freely, its mean basal value was not consistently different at the three T_b's studied under controlled conditions. The amplitude of $IENG_{ph}$ was reduced and the frequency of phrenic inspiratory cycles was increased as T_b was raised from 35.5 to 37.5°C and then to 40.2° C. Brief 100% O_2 inhalations and iv injections of dopamine produced minimal depressions of $IENG_{ph}$ amplitude in hypothermia, but pronounced although similar depressions in normothermia and hyperthermia. Iv injections of NaCN augmented f_x and $IENG_{ph}$ in dose related manner, and the relationships between both variables showed larger changes in $IENG_{ph}$ at the hypothermic and normothermic conditions when expressed in absolute terms, but not when expressed in relative terms. Thus, the chemosensory input is not consistently modified by thermal levels under controlled ventilatory conditions, but the chemosensory drive of the ventilatory output is less pronounced in hypothermia. The chemosensory input is similarly affected by varying degrees of cytotoxic hypoxia at different T_b's, but the ventilatory output is less vigorously increased in hyperthermia, pointing to a decreased reflex gain in that condition.

Key words: *body temperature, carotid body, chemosensory activity, chemoreceptors, hyperthermic hyperventilation, integrated electroneurogram, phrenic activity.*

INTRODUCTION

The chemosensory nerve discharge recorded from the carotid body superfused *in vitro* is highly dependent on the temperature of the medium when the rest of the variables are kept constant (Gallego *et al*, 1979; Alcayaga *et al*, 1993). Similarly, transient increases in the temperature of the blood circulating through one carotid produce fast increments in the frequency of chemosensory discharges (f_x) recorded from the carotid (sinus) nerve of cats (McQueen and Eyzaguirre, 1974). Otherwise, increasing the temperature of blood perfusing the vascularly isolated carotid bodies of dogs leads to transient reflex increases in breathing (Bernthal and Weeks, 1939).

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In spite of the above, recent observations show a significant but moderate contribution of the peripheral chemoreceptors to the ventilatory adjustment produced by sustained hyperthermia (Fadic *et al*, 1991). Furthermore, only slight increases in f_x were observed when raising body temperature in the spontaneously breathing cat (Loyola *et al*, 1991). Since in the whole animal, in steady-state conditions, the ventilatory regulation is maintained by homeostatic closed loops, the resultant hyperthermic hyperventilation may reduce the levels of the chemical stimuli acting upon the carotid and aortic bodies, and partially cancels the direct hyperthermic effect on chemoreceptor activity (Eyzaguirre and Zapata, 1984). Alternatively, it is possible that the hyperthermia could modulate the central gain of the chemosensory input on the inspiratory neurons. In fact, hyperthermia still induces a strong tachypnea after bilateral carotid neurotomy (Fadic *et al*, 1991).

The experiments reported here were intended to answer the apparent discrepancy in the above results. We studied the contribution of the peripheral chemoreceptors to the efferent phrenic nerve activity in the cat anesthetized with sodium pentobarbitone, paralyzed and artificially ventilated. In this preparation, the ventilatory regulation depends on the pump imposed ventilation, which allows the levels of the respiratory gases to be maintained or modified, independently of the phrenic neural output. Furthermore, sodium pentobarbitone modifies the homeothermic regulation, making the body core temperature (T_b) more dependent on environmental temperature changes (Refinetti and Carlisle, 1989).

Observations on the afferent input from arterial chemoreceptors were restricted to the chemosensory afferences from the carotid bodies. This is based on the fact that the carotid bodies play a predominant role in the excitation of ventilatory reflexes induced by cytotoxic hypoxic stimulation (Serani and Zapata, 1981) and in the chemosensory drive of ventilation under resting conditions (Eugenin *et al*, 1989). It must be noted that the section of one glossopharyngeal nerve, required for recording from the cut carotid nerve, does not modify significantly the basal ventilatory variables in pentobarbitone anesthetized cats (Eugenin *et al*, 1990).

METHODS

Experiments were performed on 12 adult male cats, weighing 2.96 ± 0.16 kg (mean ± SEM), and anesthetized with sodium pentobarbitone 40 mg/kg, ip. Additional doses (12 mg iv) were given when necessary to maintain a light level of surgical anesthesia (stage III, plane 2) ascertained by the absence of withdrawal reflexes to strong pressure on the paws, with persistence of patellar reflexes.

The cats were placed in supine position. The body core temperature (T_b) was monitored continuously through a thermistor probe introduced 50 mm into the rectum and connected to a tele-thermometer. The initial rectal temperature was 37.4 ± 0.2°C. The T_b was then maintained successively at steady-state values of 35.5, 37.5 and 40.2 ± 0.2°C, with a regulated heating pad, placed under the cat, and controlled automatically by a thermoregulator driven from the probe. To attain the hyperthermic condition, additional heat was provided by an overhead lamp (20 cm above the abdominal wall) controlled by a dimmer. Care was taken not to exceed 50° C of surface temperature at the exposed sites of the skin. The experiments were performed under the following prevailing conditions: room temperature 28.3± 1.4° C, relative humidity 62.5 ± 1.5 %, atmospheric pressure 740.3 ± 0.2 Torr.

The trachea was cannulated *per os* with a flexible tube (3.8 mm ID, 4.4 mm OD). A fine (PE-10) tubing was introduced deep into the tracheal cannula for continuous sampling of air and recording of the tracheal CO_2 pressure (P_TCO_2) through an infrared gas analyzer. Alveolar ventilation was estimated from breath-by-breath end-tidal CO_2 pressure ($P_{ET}CO_2$), calculated after subtracting the H_2O pressure in moist tracheal air (47 Torr) from the barometric pressure.

The right saphenous vein was cannulated for administration of drugs. Systemic arterial pressure was recorded from the right femoral artery through a cannula (PE-100) filled with heparin 50 IU/ml in saline solution, and connected to a pressure transducer. Heart rate was counted electronically using a tachograph triggered by the arterial pressure pulse.

The cats were paralyzed by iv administration of alcuronium chloride (100-200 μg/kg

initially, and subsequently 50-100 μg/kg every 30 min) and artificially ventilated with a positive-pressure ventilator. The tracheal cannula was connected to a Y-shaped tube. One arm was connected to the ventilator through a heated Fleisch pneumotachograph (number 00) and to a PT-5 volumetric pressure transducer for reading inspiratory flow. Tidal volume (V_T) was obtained by electronic integration of the flow signal. The other end of the Y-tube was connected to a piece of flexible tubing ending at the bottom of a bottle containing water (5-8 cm height) to allow expiration and escape of the extra inspiratory air. At the beginning of the artificial ventilation, the inspiratory volume was fixed to obtain a $P_{ET}CO_2$ value similar to the previous one, recorded during spontaneous breathing. The expiratory side of the system was blocked for 1 or 2 cycles from time to time (5 min) to produce augmented inspirations (simulated gasps), intended to maintain an adequate alveolar surfactant. Since hyperthermia raised $P_{ET}CO_2$, the frequency of the ventilator was augmented to keep the $P_{ET}CO_2$ low.

The physiological variables were displayed on a polygraph and a multiple beam oscilloscope. Raw signals were stored on a digital video system (through an analog-digital converter) for latter analyses.

Recordings of afferent and efferent fibers

The right carotid and phrenic nerves were exposed through a lateral longitudinal incision in the neck. The branch of the phrenic nerve arising from the C5 root was dissected free of the epineurium, cut caudally, placed on a pair of bipolar Pt-Ir electrodes and covered with warm mineral oil. The efferent neural discharges were preamplified, amplified, full wave rectified ($RENG_{ph}$) and integrated ($IENG_{ph}$). The efferent signals were also fed to an amplitude window discriminator and the instantaneous frequency ($\dot{f}_{ph}$) of the standardized pulses was obtained electronically.

In five experiments, the carotid (sinus) nerve was dissected and cut, and the ipsilateral ganglioglomeral nerves were also cut. Barodenervation was performed through section and crush of nerve filaments between the emergence of the internal carotid artery and the carotid body (Zapata *et al*, 1969). After excision of the epineurium, the nerve was placed on a pair of Pt-Ir electrodes and covered with warm mineral oil. The signals were preamplified, amplified, passed through a 50 Hz filter and fed to an electronic amplitude discriminator which allowed selection of action potentials of given amplitudes above the baseline noise. The frequency of the resulting standardized chemosensory pulses (f_x) was counted every second by means of an electronic counter-printer system and obtained as instantaneous rate through a frequency meter.

Recordings of the carotid afferent and phrenic efferent nerves on the same side is justified by the fact that ventilatory chemoreflexes are not lateralized (Berger and Mitchell, 1976).

Testing of chemosensory drive and chemoreflex responses

The contribution of the peripheral chemosensory drive to the phrenic nerve activity was estimated from the changes in amplitude of the $IENG_{ph}$, resulting from the withdrawal of the chemosensory impulses caused by exposure to 100% O_2 (Dejours, 1963) for 1 min through the ventilator, and by iv injections of dopamine-HCl (1-50 μg/kg; prepared in ascorbic acid 1 mM in saline) (Zapata and Zuazo, 1980).

Chemoreflexes sensitivity and reactivity were assessed by studying the phrenic neural responses to increasing iv injections of NaCN (0.5 to 100 μg/kg) and by administration of 100% N_2 for 10-15 s through the ventilator. The tests were repeated at the three T_b's at steady-state conditions.

Data analyses

Ventilatory variables were measured and averaged over periods of 1 min during steady-state thermoregulatory and ventilatory conditions. Respiratory frequency (f_R) was measured from the phrenic burst frequency. Considering that the $IENG_{ph}$ is the neural equivalent of tidal volume (V_T), a neural inspiratory minute volume index ($\dot{V}_{ph}$) was estimated by multiplying $IENG_{ph}$ amplitude by f_R.

Results are expressed as means ± SEM's or as percentages of their respective values. Statistical differences for multiple dependent

samples were assessed by either Quade's or Friedman's test, whichever the most appropriate, followed by paired comparisons through Conover's tests (Theodorson-Norheim, 1987).

To compare data from different experiments, the cyanide dose-response curves were fitted to symmetrical sigmoidal functions (De Lean *et al*, 1978). This method provides a basis for pooling data from separate experiments, and allows testing the characteristics which are shared by various curves. The data points were adjusted according to the following logistic expression for responses expressed in percentages:

$$R = \max R + [100 - \max R] / [1 + (D/ED_{50})^S]$$

where: **R** = response; **max R** = maximal response; **D** = arithmetic dose; $\mathbf{ED_{50}}$ = median effective dose; **S** = slope factor determining the steepness of each curve. The curves were fitted through a computer program based on a simplex algorithm (Johnston, 1985). The goodness of each fit was tested using an ANOVA and by the correlation coefficient (**r**), calculated by dividing the variance of the theoretical value by that of the experimental value.

RESULTS

Effect of thermal conditions on resting respiratory variables

Figure 1A shows a summary of the mean values of $P_{ET}CO_2$ observed at steady-state T_b's of 35.5, 37.5 and 40.2 ± 0.2° C in the 7 cats with intact carotid nerves. Before adjustments in the frequency of the pump, the $P_{ET}CO_2$ increased slightly from 33.6 ± 2.2 Torr at 35.5° C to 37.0 ± 2.5 Torr at 37.5° C ($p < 0.05$), and then considerably to 56.8 ± 7.9 Torr when T_b was raised to 40.2° C ($p < 0.01$). In order to maintain $P_{ET}CO_2$ relatively low, the frequency of the pump ventilator (f_p) was raised from 22.0 ± 2.8 min^{-1} to 28.7 ± 3.1 min^{-1} at the higher T_b level (fig 1B), which resulted in a reduction in $P_{ET}CO_2$ to 33.5 ± 1.3 Torr, a value not significantly different from those observed at lower temperatures (fig 1A). In 3 out of the 7 experiments, we avoided the potentially noxious effects of the hypercapnia and acidosis provoked by raising T_b to 40.2° C by progressively increasing the frequency of the ventilator during the warming procedure.

The mean amplitude of the $IENG_{ph}$ recorded at basal conditions was considerably reduced at the normothermic level in comparison with the hypothermic level, and a further but small decrease occurred when reaching the hyperthermic isocapnic condition (fig 2A). On the contrary, the respiratory neural frequency (f_R) increased significantly when T_b and f_p were elevated (fig 2B). As a result of the progressive decreases in magnitude of the phrenic inspiratory bursts and the reciprocal increases in f_R observed while increasing T_b, the neural minute volume ($\dot{V}_{ph}$) was not significantly modified by thermal changes (fig 2C).

The large increase in f_R observed in 4 cats when augmenting f_p at 40.2° C (fig 2B) is

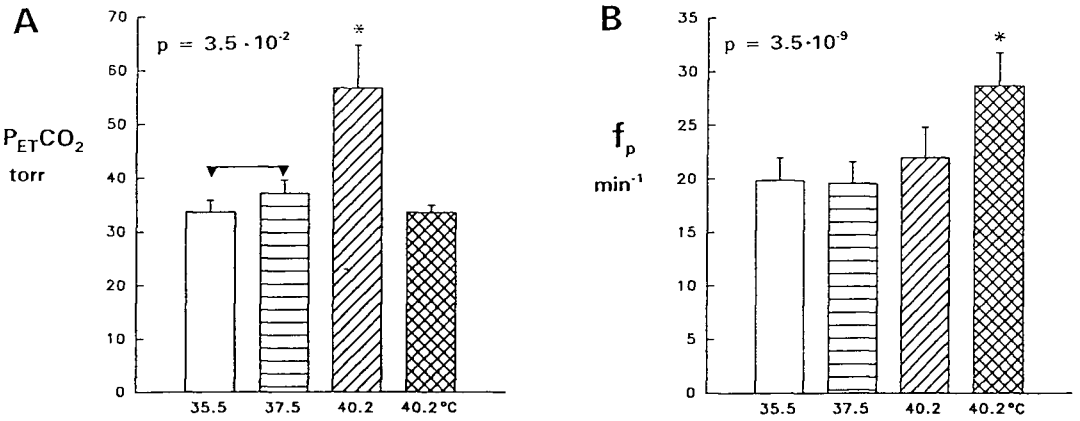

Fig 1. Means + SEM's of (A) end-tidal CO_2 pressure ($P_{ET}CO_2$) and (B) frequency of pump ventilator (f_p) at three different steady-state T_b's in 7 cats with intact carotid nerves. Open bars, at 35.5° C; horizontally striped bars, at 37.5°C; hatched bars, at 40.2° C before adjusting f_p to control $P_{ET}CO_2$ in 4 cats; cross-hatched bars, at 40.2° C after raising f_p to maintain $P_{ET}CO_2$ low. Statistical significance of multiple comparisons (insets, overall p values from Friedman's tests, n = 7), followed by paired comparisons (asterisks, significantly different from all the rest at $p < 0.01$; between arrows, difference at $p < 0.05$). Quade's tests applied for comparisons of hypercapnic hyperthermic cats with other conditions (n = 4).

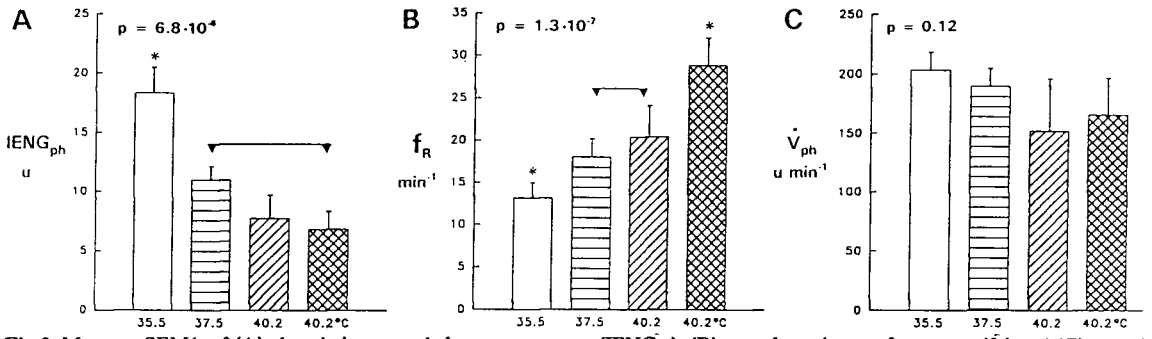

Fig 2. Means + SEM's of (A) phrenic integrated electroneurogram ($IENG_{ph}$), (B) neural respiratory frequency (f_R) and (C) neural inspiratory minute volume ($\dot{V}_{ph}$) at 3 different steady-state T_b's in 7 cats with intact carotid nerves. Bars and statistical analyses, as in Figure 1.

clearly the result of the entrainment of the phrenic inspiratory bursts by the frequency of the pump ventilator, since it is associated to a fall in basal $P_{ET}CO_2$ (fig 1A) and occurs without change in T_b. The phrenic bursts were also entrained to the ventilator rate at the other T_b's, as illustrated in figure 3. There, an abrupt increase in f_p from 14 cycles/min to 22 cycles/min produced an immediate change in f_R from 14 "neural" breaths/min to 22, which was also abruptly reversed when returning to the initial conditions. This indicates that neural bursts were all time entrained by the lung inflations imposed by the pump. Furthermore, the abrupt transition to a higher frequency of ventilatory pumping and thence of phrenic bursts is followed by a slow progressive decrease in the strength of the inspiratory phrenic discharges estimated by the $IENG_{ph}$ (fig 3). Since in all experiments f_p was increased, either progressively or in steps, during the warming procedure, the entrainment of phrenic activity may contribute to the observed increase in f_R and decrease in $IENG_{ph}$ when passing from the hypothermic to the normothermic and hyperthermic levels.

In some artificially ventilated cats we observed the appearance at regular intervals of biphasic bursts of phrenic discharges within a given cycle, the intensity and timing of the initial phase being similar to those of the remaining inspiratory cycles, but surmounted by an extraordinary burst of higher instantaneous frequency. These two phases are clearly discernible in the f_{ph} and $RENG_{ph}$ recordings, but they add in the $IENG_{ph}$ to exhibit a motoneuronal burst of much larger strength than the rest. These augmented inspiratory bursts were more commonly seen

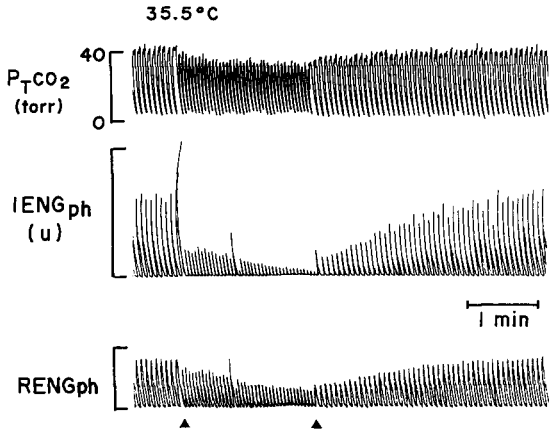

Fig 3. Changes in tracheal pressure of CO_2 (P_TCO_2; first row), phrenic integrated electroneurogram ($IENG_{ph}$; second row) and phrenic rectified electroneurogram ($RENG_{ph}$; third row) in response to switching the pump ventilator from 14 to 22 cycles/min (first arrow head) and then back to 14 cycles/min (second arrow head). Cat with both carotid nerves intact and thermostabilized at 35.5° C.

at the higher T_b (see last rows in figs 7 and 8) or they increased in frequency when raising T_b from 37.5 to 40.2° C.

Effect of thermal conditions on the response to NaCN

Figure 4 illustrates the effects of steady levels of T_b on the phrenic nerve responses to two low-to-intermediate doses of NaCN (5 and 10 µg/kg, iv) in one cat with both carotid nerves intact. These injections increased the magnitude of the $IENG_{ph}$ and the duration of the inspiratory bursts at 35.5 and 37.5° C, but did not elicit any response at 40.2° C. The increase in the $IENG_{ph}$ in response to 10 µg NaCN is not associated to an augmented instantaneous rate of phrenic bursts (f_{ph}), thus implying that

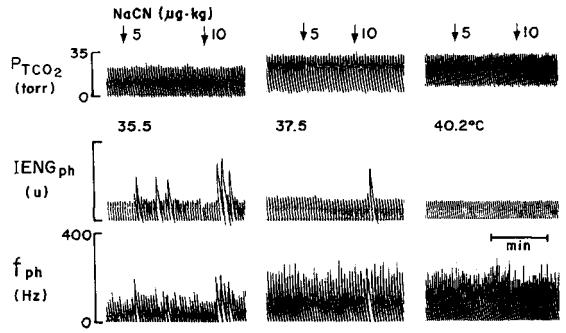

Fig 4. Phrenic neural responses to NaCN (5 and 10 µg/kg, iv) at three different steady-state T_b's in a cat with intact carotid nerves. P_TC_{O2}, tracheal CO_2 pressure (first row); $IENG_{ph}$, phrenic integrated electroneurogram, in arbitrary units (second row); $\dot{f}_{ph}$, instantaneous frequency of phrenic discharges (third row).

the enhanced phrenic output was not due to an increased maximal frequency of motoneuronal discharges but to a prolongation of such discharges, as evidenced by the configuration of $\dot{f}_{ph}$ during the affected cycle.

A systematic study of the phrenic responses elicited by increasing doses of NaCN (0.5 to 100 µg/kg) was performed with the data obtained from 7 cats with intact carotid nerves. For this study, the $P_{ET}CO_2$ at 40.2° C was kept at a similar value as those observed at 35.5 and 37.5° C. Figure 5 shows the fitting to a sigmoidal function of the maximal $IENG_{ph}$ responses (in percentages of basal activity) produced by NaCN at the three steady-state T_b's levels. Statistically significant differences were observed in only one of the three parameters defining the dose-response curves (Table I). The maximal (theoretical) reactivity and the slope factor did not differ between the three thermal conditions, but the mean effective dose was displaced to the right by nearly one order of magnitude at 40.2° C.

The pattern of phrenic nerve discharges was much modified by cyanide in some occasions. Figure 6 illustrates the effects of large doses of NaCN (20 to 100 µg/kg iv) and those of evoked gasps (see Methods) on the cycles of phrenic discharges. NaCN prolonged the duration of the inspiratory discharge of the phrenic nerve and thus augmented considerably the amplitude of $IENG_{ph}$. A peculiar pattern of interlocked bursts of phrenic cycles (one very prolonged and thus much enhanced and one or two with normal duration but increased maximal instantaneous frequency) was triggered by the two first injections of cyanide.

A simpler and continuous pattern of prolonged but paused phrenic discharge was initiated by the last injection of this agent. Simulated gasps (lung inflations along two cycles uninterrupted by expirations, thus doubling V_T), on the contrary, switched-off the augmented phrenic output, returning the respiratory pattern to basal conditions.

Effect of thermal conditions on the responses to 100% O_2 and dopamine

Figure 7 illustrates the transient depression of efferent phrenic neural activity in response to

TABLE I

Parameters defining dose-response curves for changes in phrenic activity evoked by iv injections of NaCN in 7 cats with intact carotid nerves

T_b °C	35.5	37.5	40.2	p
max R	258.7	264.3	262.3	0.093
ED_{50}	15.1	9.2	89.9 *	0.006
S	1.3	1.6	0.9	0.185
r	0.997	0.998	0.969	-

r, correlation coefficients for fitting the curves to sigmoidal functions.
p, overall significance, after Friedman's tests.
* $p < 0.01$, *vs* the rest, after Conover's tests.

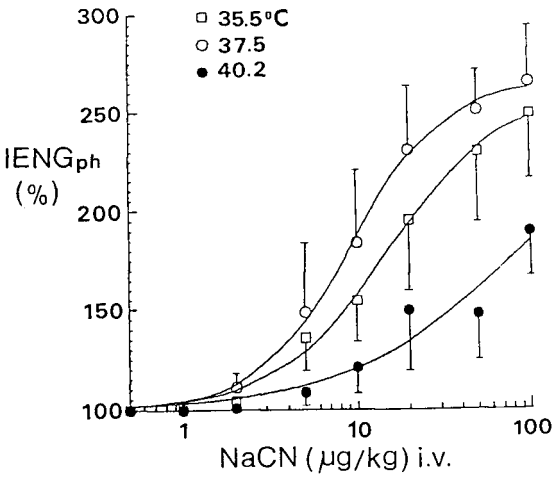

Fig 5. Dose-response curves for changes in amplitude of phrenic integrated electroneurogram ($IENG_{ph}$) elicited by increasing doses of NaCN iv at 3 different steady-state T_b's, obtained from 7 cats with intact carotid nerves. $P_{ET}CO_2$ values: 31.5 ± 1.8 torr at 35.5°C (open squares), 32.6 ± 1.6 torr at 37.5° C (open circles) and 31.6 ± 1.2 torr at 40.2° C (filled circles). Responses (means + or - SEM's) expressed as percentages of basal values. SEM's are only depicted to illustrate dispersion of values, since statistical analyses were based on ranks (Friedman's tests).

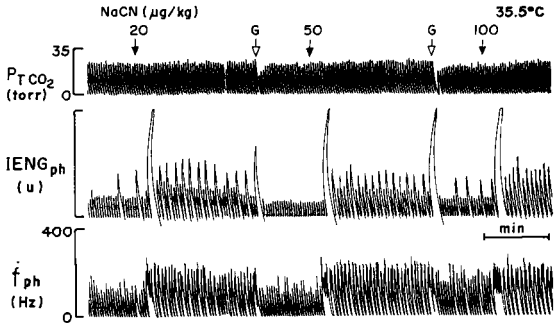

Fig 6. Effects of large doses of NaCN (20-100 µg/kg iv, filled arrows) and simulated gasps (G, open arrows) on phrenic efferent activity in a cat with intact carotid nerves, at 35.5° C. P_TCO_2, tracheal pressure of CO_2 (first row); $IENG_{ph}$, phrenic integrated electroneurogram (second row); f_{ph}, instantaneous frequency of phrenic discharges (third row).

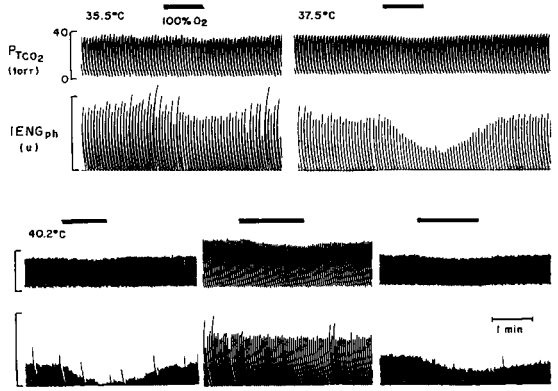

Fig 7. Transient effects of 1 or 1.5 min periods of 100% O_2 inhalation on phrenic neural activity at three different T_b's. P_TCO_2, tracheal pressure of CO_2 (first rows); $IENG_{ph}$, phrenic integrated electroneurogram, in arbitrary units (second rows). At 40.2°C, response tested at two levels of $P_{ET}CO_2$; note there the presence of the neural equivalent of spontaneous gasps.

breathing 100% O_2 at the three T_b's levels and at two different $P_{ET}CO_2$ values. At 35.5°C, 100% O_2 inhalation reduced only moderately the $IENG_{ph}$ (upper left panel). A more pronounced decrease of the integrated neural output was observed at 37.5° C (upper right panel). When the f_p was raised at 40.2° C to maintain $P_{ET}CO_2$ low (lower left panel) an intense transient reduction of the amplitude of the $IENG_{ph}$ was observed in response to hyperoxia. However, when the frequency of the ventilator was reduced and both basal $P_{ET}CO_2$ and basal amplitude of $IENG_{ph}$ augmented, 100% O_2 inhalation was ineffective to reduce the phrenic output (lower middle panel). After the return to the previous conditions, 100% O_2 inhalation again diminished the amplitude of phrenic nerve inspiratory bursts (lower right panel).

Figure 8 illustrates the phrenic neural response to the iv administration of dopamine (2-10 µg/kg). These doses were selected because they did not elicit considerable pressor responses (less than 10 Torr). Large doses of dopamine (20-100 µg/kg) produced marked hypertensive reactions (mean arterial pressure went up to 200 Torr or more), which in turn may depress the neural phrenic output since ventilatory depressant effects of large doses of dopamine in spontaneously breathing cats are still observed after section of the carotid and aortic nerves (Zapata and Zuazo, 1980), because of rise in intracisternal cerebrospinal fluid pressure (Serani *et al*, 1981).

The amplitude and duration of the $IENG_{ph}$ depressant response to dopamine was markedly augmented at 37.5°C as compared with that observed at 35.5° C. At 37.5° C, the doses of 5 and 10 µg/kg briefly silenced the phrenic nerve discharges (fig 8). At 40.2° C, the depressant effects of dopamine upon phrenic output persisted, but they were not more pronounced than those observed at 37.5° C.

Effects of thermal conditions on basal and evoked chemosensory activities

Figure 9 summarizes the effects of changes in steady-state T_b's on the mean frequencies

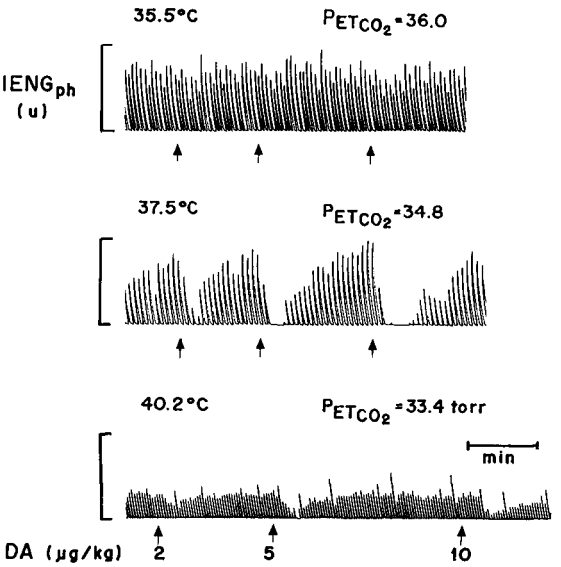

Fig 8. Phrenic neural responses to iv injections of dopamine-HCl (2, 5 and 10 µg/kg, at arrows) at three different T_b's in a cat with intact carotid nerves. $IENG_{ph}$, phrenic integrated electroneurogram, in arbitrary units. Note the presence of neural equivalent of spontaneous gasps, at 40.2°C.

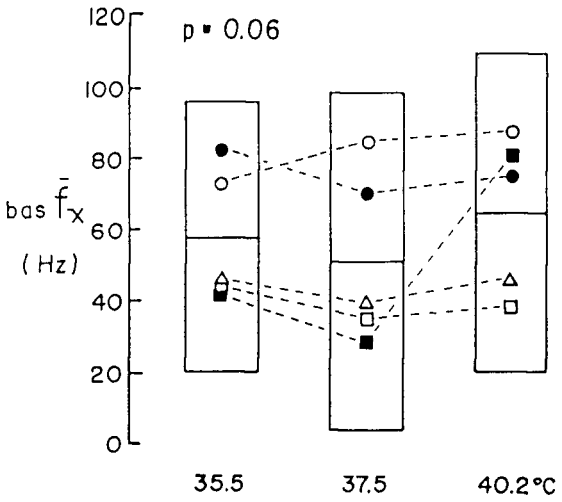

Fig 9. Frequencies of basal mean chemosensory discharges (bas $\dot{f}_x$) recorded from one cut carotid nerve in 5 cats studied at steady-state T_b's of 35.5, 37.5 and 40.2°C. Each symbol is the mean of 60 consecutive counts of 1-s periods in a different cat. Rectangles, 99% normal distribution of the grand means (horizontal lines at the middle of the rectangles). p at inset, statistical significance of multiple comparisons by Friedman's test.

of basal chemosensory activity in 5 cats in which one carotid nerve was cut for recording. These basal values were obtained from 1 min recordings under isocapnic conditions. Although in 5 experiments the mean f_x's increased between 37.5 and 40.2° C and in 4 of them decreased between 35.5 and 37.5° C, the differences observed between the grand means did not reach statistical significance (Friedman's test; $p = 0.06$).

To ascertain the degree of carotid chemoreceptor excitation dependent on the normoxic conditions at different T_b's, we studied the falls in f_x resulting from 100% O_2 inhalations in the 5 animals in which carotid chemosensory discharges were recorded under isocapnic conditions. The maximal transient reductions in f_x induced by hyperoxic tests were not significantly different at the three different T_b's (Quade's test; $p = 0.25$).

Figure 10 illustrates the changes in fx induced by increasing doses of NaCN given iv at the three steady-state T_b's. In spite of the fact that bas f_x was enhanced at the hyperthermic level in this animal, no changes in maximal reactivity, slope or mean effective dose were observed between the three thermal levels. Maximal reactivity in absolute terms was different from one animal to other because of the different number of chemosensory fibers recorded from their respective carotid nerves. However, no significant changes in dose-response curves between different T_b's were also observed in three other cats. In only 1 out of the 5 cats, the maximal reactivity was considerably reduced at 40.2° C, nearly to one third of that recorded at 35.5° C, with an intermediate value reached at 37.5° C, but this observation may be a consequence of a progressive reduction in the number of active fibers within the dissected carotid nerve along the several hours of recording.

Relationship between carotid chemosensory and phrenic nerve discharges

Figure 11 illustrates the effects of several increasing doses of NaCN (0.5 to 100 μg/kg, iv) on the carotid chemosensory frequency and the integrated phrenic discharges in one cat (with one carotid nerve sectioned) studied at three different T_b levels. NaCN increased f_x and $IENG_{ph}$ amplitude in a dose-related manner, with a clear cut correlation in intensities and timing, particularly evident at 37.5° C (fig 11B).

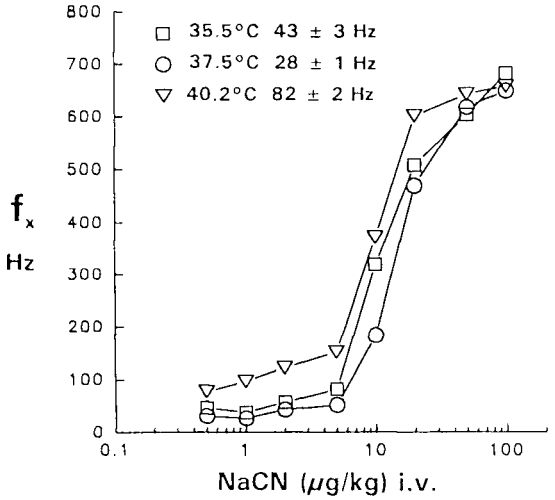

Fig 10. Changes in the frequency of chemosensory impulses (f_x) recorded from one cut carotid nerve in response to increasing doses of NaCN given iv to a cat at 35.5°C (squares), 37.5°C (circles) and 40.2°C (triangles). Inset, means ± SEM's of basal chemosensory frequency. For the three conditions, frequencies evoked by NaCN 5 μg/kg and higher doses were significantly above the 95% normal distribution of their respective basal means.

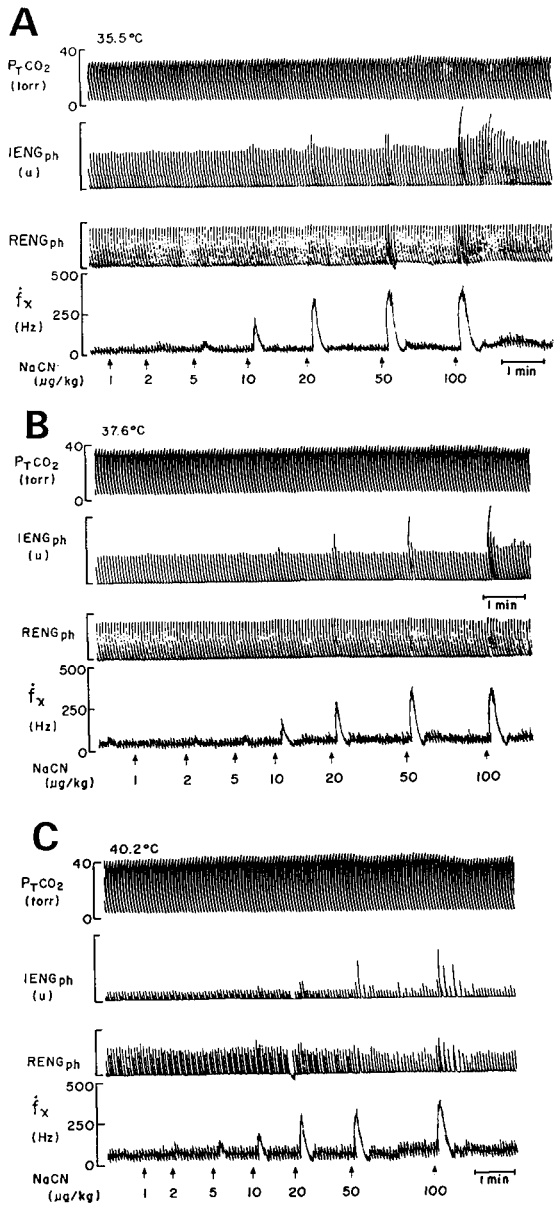

Fig 11. Carotid chemosensory and phrenic efferent responses to several doses of NaCN (1 to 100 µg/kg, iv) at 35.5°C (A), 37.5°C (B) and 40.2°C (C), in a cat with one carotid nerve sectioned. P_TCO_2, pressure of tracheal CO_2 (first rows); $IENG_{ph}$, phrenic integrated electroneurogram (second rows); $RENG_{ph}$, phrenic rectified electroneurogram (third rows); $\dot{f}_x$, instantaneous frequency of chemosensory discharges (fourth rows).

In spite of the above, time correlations between the discharges of chemosensory afferents and phrenic efferents were sometimes distorted. Thus, figure 12 displays at higher speed the responses to NaCN 100 µg/kg iv at 35.5° C to show that the drug increased f_x, the duration of the burst of phrenic discharges (see $RENG_{ph}$ recording) and the amplitude of the $IENG_{ph}$. The chemosensory excitation declined rapidly, while phrenic efferent activity remained elevated for a prolonged period. Here, the rectified ENG shows that the early and late increases in amplitude of the integrated ENG were not due to higher discharge frequencies of phrenic motoneurons, but to prolongations of their bursts of inspiratory discharges.Contrariwise, less intense increases of phrenic nerve bursts in response to NaCN injections observed at 40.2° C (fig 11C) were associated to prolongations of expiratory silences.

Figure 13 shows the relationships between the carotid chemosensory afferent and the phrenic efferent activities at the three steady-state T_b's levels, but at similar $P_{ET}CO_2$ levels. These data were obtained during chemosensory excitations induced by increasing doses of NaCN and 100% N_2 tests, and during transient depressions of chemosensory drive by dopamine (5-10 µg/kg) and 100% O_2 tests. When the $IENG_{ph}$ is expressed in arbitrary units (but derived from recordings at the same amplification), a gross inspection gives the

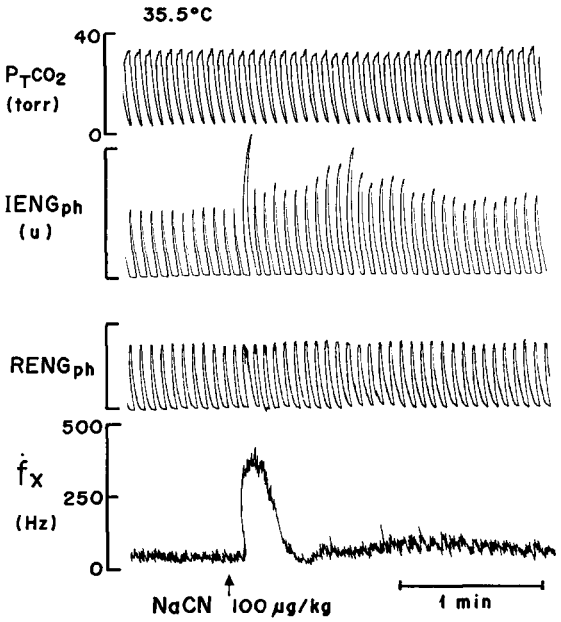

Fig 12. Response of carotid afferents and phrenic efferents to a large dose of NaCN (100 µg/kg, iv) at 35.5°C in a cat with one carotid nerve sectioned. P_TCO_2, tracheal pressure of CO_2 (first row); $IENG_{ph}$, phrenic integrated electroneurogram (second row); $RENG_{ph}$, phrenic rectified electroneurogram (third row); $\dot{f}_x$, instantaneous frequency of carotid chemosensory discharges (fourth row). Display at higher speed of recording from upper right corner of Fig 11.

impression that the same chemosensory input produced a smaller phrenic efferent activity at the higher T_b (fig 13A). However, when $IENG_{ph}$ was expressed as a percentage of the basal values, the difference disappeared (fig 13B). Thus, the same chemosensory input produced a proportional phrenic output above baseline. In fact, the amplitude of the basal $IENG_{ph}$ was strongly reduced at 40.2° C, explaining the maintenance of the ratio evoked/basal $IENG_{ph}$ activities at different temperatures.

DISCUSSION

Observations on the sensitivity of carotid bodies to local thermal changes, originated from different laboratories and referred to in the Introduction, indicate that these organs are extremely sensitive detectors of fast changes in temperature and that their basal levels of neural afferent discharges are positively correlated with the steady levels of local temperature. The high energies of apparent activation (μ), high thermal coefficients (Q_{10}) and high thermal gains (df_x/dT_b) exhibited by the rates of afferent discharges of the carotid bodies indicate that they fulfill the criteria for being considered as potential thermosensors (Alcayaga *et al*, 1993). Furthermore, their profuse vascularization and intimate relation to the blood make them suitable to serve as adequate detectors for changes of the blood temperature. Being the temperature of the blood at deep structures the best representative of body core temperature (T_b), the functioning and location of the carotid bodies make them ideally suited to serve as sensors for changes in the steady-state levels of T_b.

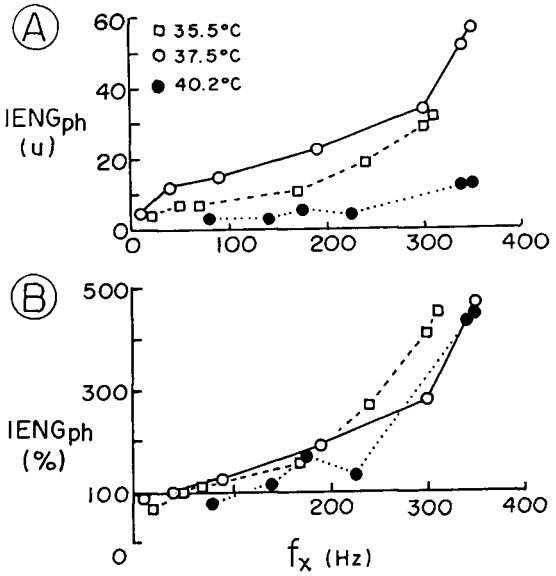

Fig 13. Relationships between afferent carotid chemosensory and efferent phrenic activities in a cat at three different T_b's. Abscissae, frequency of chemosensory discharges (f_x). Ordinates, phrenic integrated electroneurogram ($IENG_{ph}$), expressed in arbitrary units at the same amplification (A) and as percentages of basal values (B). Increases in both variables were produced by NaCN injections at different doses; decreases (below 100%, basal values), by 100% O_2 tests and dopamine injections.

Since the carotid bodies play a predominant role in the regulation of respiration at resting conditions and during adaptation to changes in the chemical composition of the blood (Fitzgerald and Lahiri, 1986), it was pertinent to ask if these receptors should participate in the important adjustments in pulmonary and alveolar ventilation produced by thermal challenges.

In awake cats, the mean rectal temperature of 38.8° C is maintained at environmental temperatures of 20 to 30° C; increasing the ambient temperature above 30° C induces progressively higher rectal temperatures of up to 40.5° C, and increased respiratory frequency, panting appearing when the environment surpasses 41° C (Adams *et al*, 1970; Bonora and Gautier, 1989). Also peak rectal temperatures of 40.7 ± 0.1° C had been recorded in unanesthetized cats injected with synthetic pyrogens (Morilak *et al*, 1987). Since ventilation is important for heat dissipation in cats, because of their furred skin and restriction of sweat glands to toes and footpads, this species is well suited to study ventilatory reactions to variations in body temperature, and the possible contribution of carotid body chemoreceptors as thermal afferents.

Previous observations from this laboratory (Fadic *et al*, 1991) indicate that increasing T_b in the pentobarbitone anesthetized cat, by moderately heating the skin surface, provoked pulmonary and alveolar hyperventilation. This was mostly mediated by increasing frequencies of respiration and spontaneous gasps, with a minor contribution of V_T. The last one was clearly dependent on the indemnity of the carotid nerves, while pronounced increases in f_R were still observed after bilateral section of

the carotid nerves. Similarly, von Euler *et al* (1970) observed increases in both V_T and f_R when raising T_b from 37.1° to 39.6° C in pentobarbitone anesthetized cats, the tachypnea being preserved after bilateral vagotomy, which included interruption of the chemosensory afferences from the aortic bodies. Studies on pentobarbitone anesthetized cats (Grunstein *et al*, 1973) and in urethane anesthetized rabbits (Kobayasi and Yamaguchi, 1981) had shown that f_R was extremely dependent on T_b in the range of 35° C to 41° C, resulting in fairly high Q_{10} values (6.1 and 5.3, respectively), persisting after bilateral vagotomy.

The modest role played by carotid bodies afferents in the ventilatory adaptation to slow changes in T_b, observed in spontaneously breathing cats (Fadic *et al*, 1991), may be ascribed to the alveolar hyperventilation itself caused by hyperthermia, the resulting changes in blood gases counteracting the stimulating local effects of the increased blood temperature upon carotid bodies. Nevertheless, the present results, obtained in artificially ventilated cats in which these changes in blood may be controlled and suppressed, indicate that the reflex effects elicited by chemosensory stimulants of the carotid and aortic bodies are not potentiated by increasing T_b above normal levels. Results indicate that the correlation between changes in the frequency of chemosensory afferents and those in the strength of the ventilatory output (phrenic efferents) is well maintained at the three levels of temperature studied: moderate hypothermia, normothermia and mild hyperthermia.

Considering $IENG_{ph}$ as the neural output equivalent of V_T, the decrease in $IENG_{ph}$ observed at 37.5° C when compared to that recorded at 35.5° C (fig 2A) may be related to the decrease in V_T occurring in spontaneously breathing pentobarbitone-anesthetized cats when rewarming from 28 to 38° C (Gautier and Gaudy, 1986). Similarly, the further decrease in $IENG_{ph}$ observed when reaching 40.2° C under isocapnic conditions (fig 2A) may be compared to the decrease in V_T occurring in unanesthetized cats when T_b is displaced from 38.6 to 39.4° C by external warming (Bonora and Gautier, 1990). Both decreases in V_T were associated to concomitant increases in f_R (Bonora and Gautier, 1990; Gautier and Gaudy, 1986), as also observed in our artificially ventilated cats (fig 2B).

With regard to resting ventilation, the basal levels of carotid chemosensory discharges maintain a certain degree of tonic reflex excitation of ventilatory centers ("chemosensory drive"), demonstrated by the immediate, transient decreases in tidal volume in response to breathing 100% O_2 for a few seconds (Dejours, 1963) or iv injections of dopamine (Zapata and Zuazo, 1980), as consequence of the sudden withdrawal of carotid chemosensory discharges, briefly silenced by these maneuvers (Leitner *et al*, 1965; Llados and Zapata, 1978). Therefore, if a rise in blood temperature should increase the basal frequency of chemosensory discharges, the chemosensory drive upon resting ventilation should also be enhanced. In humans using heated flying suits, an increase in rectal temperature of 1.6° C enhanced the chemosensory drive upon ventilation, as revealed by the more pronounced transient ventilatory depression in response to 2 breaths of pure O_2 (Jensen *et al*, 1978). In pentobarbitone anesthetized, spontaneously breathing cats, brief hyperoxic tests induced transient decreases in tidal volume and increases in $P_{ET}CO_2$ which were significantly larger at 40° C than at 37° C (Fadic *et al*, 1991). Nevertheless, present results indicate that in the artificially ventilated cat in which $P_{ET}CO_2$ is kept within eucapnic range, 100% O_2 tests and dopamine injections applied in the hyperthermic and normothermic conditions decreased the $IENG_{ph}$ amplitude more profoundly than in hypothermia (figs 7 and 8), in spite of the similar depressant effects upon chemosensory discharges exerted by these maneuvers at the three levels of T_b studied. It is noteworthy that the ventilatory drive exerted by arterial chemoreceptors was not proportionally reduced in hyperthermia, as one would expect from the increased thermal drive exerted directly upon central respiratory neurons. Similarly, in unanesthetized cats dopamine injections produce significant decreases in V_T in both normothermic and hypothermic conditions (Bonora and Gautier, 1990).

Present results also indicate that stronger ventilatory effects were evoked by milder degrees of hypoxic stimulation in cats thermostabilized at 37.5° C than in those kept

at either 35.5 or 40.2° C (figs 5 and 13A). Thus, taking together the depth of the chemosensory drive and the efficiency of the chemoreflexes, it appears that the maximal efficiency of the arterial chemoreceptors as controllers of ventilation is attained at the normal T_b of the animal. This is the kind of operation that one would expect for a thermostatic system that corrects rapidly and efficiently minor deviations from the thermal set point.

The pattern of ventilation is profoundly affected by T_b. The bradypnea of hypothermia and the tachypnea of hyperthermia result from changes in the durations of the inspiratory bursts and expiratory silences exhibited by those nerves controlling the main respiratory muscles. Present results indicate that the duration of phrenic inspiratory bursts decreases moderately while T_b is increased from 35.5° C to 37.5° C, and that both inspiratory duration and total discharges per cycle of the phrenic nerve are reduced at 40.2° C. Furthermore, different patterns of ventilation were observed in response to hypoxic stimulation at different steady levels of T_b.

It was interesting to observe that an intense chemoreceptor stimulation -induced by the largest doses of NaCN- provoked early and delayed increases in the rate of the discharges recorded from phrenic motoneurons (fig 12). The early enhancements of motoneuronal discharge cycles were correlated in time and magnitude with the concomitant increases in the frequency of carotid chemosensory discharges. However, the simultaneous analysis of rectified and integrated phrenic discharges showed longer durations of inspiratory bursts along several cycles, well beyond the transient early increases in chemosensory discharges; the potentiation of these motoneuronal discharges was not correlated with the mild delayed increase in chemosensory discharges. This is similar to an observation of Lahiri *et al* (1991) in which an intracarotid injection of 20 μg of NaCN produced a brief increase of f_x but a long-lasting increase in ventilation (see fig 2B of that report). This phenomenon reminds us the prolonged stimulation of respiration evoked by several types of brief intense stimuli, including the electrical stimulation of the carotid nerves (Millhorn *et al*, 1980). Thus, it is possible that carotid body stimulation –under certain conditions– evokes not only a reflex concomitant increase in ventilatory output, but also resets the timing components (off-switch) of the central generator of respiration.

An interesting observation was the appearance at regular intervals in some of these artificially ventilated cats of biphasic bursts of phrenic neural discharges. The regularity of these periodic cycles and their biphasic pattern reveal that they correspond to the neural equivalent of spontaneous gasps, also named inspiratory augmenting responses, sighs or periodic deep breaths (Bartlett, 1971). The electromyographic correlate of these augmented breaths is an increased post-inspiratory inspiratory activity in the diaphragm (van Lunteren *et al*, 1986), which is in turn evoked by an augmentation phase in phrenic nerve activity, initiated close to the crest of the normal inspiratory burst (Cherniack *et al*, 1991), when these variables are recorded in spontaneously breathing anesthetized cats. However, to our knowledge, this is the first description of these phrenic ENG equivalents to spontaneous gasps in artificially ventilated animals and the first evidence of their periodic occurrence in this controlled condition. Their most common incidence or higher frequency at hyperthermic levels (see records in figs 7 and 8) is coincident with the increase in spontaneous gasps observed in spontaneously breathing cats in hyperthermia (Fadic *et al*, 1991) and may be explained by the modulatory role exerted by carotid chemosensory efferents upon the pulmonary vagal reflex responsible for these augmented breaths (Zuazo and Zapata, 1980).

In summary, observations here reported indicate that in the normoxic eucapnic animal, the most pronounced influence of arterial chemoreceptors operating as thermosensors controlling ventilation is observed within the normothermic range.

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