Cooling of Ventral Medullary Intermediate Areas and Respiration in the Cat

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Summary We sought to address the contribution of the ventral medullary intermediate (I) areas to respiratory regulation in the close loop condition. The experiments were done on anesthetized, spontaneously breathing cats. The transient and steady-state neural respiratory output responses to longlasting cooling of the I areas and to vagotomy and hyperoxia performed during the cooling period were investigated. Cooling of the I areas resulted in an initial transient inhibitory response followed by respiratory output increase in the steady-state, provided other respiratory inputs were maintained intact. This investigation calls into question the crucial role of the I areas in regulation of respiration in the close loop condition.

Key words: central chemoception, central respiratory controller, phrenic nerve.

The input from the intracranial chemoceptive structures to the central respiratory controller (CRC) has been shown in many reports to be reversibly blocked by bilateral cooling of the I areas on the ventral medullary surface (VMS) (see e.g. BRUCE and CHERNIACK, 1987). In the open loop condition (paralyzed animal on constant artificial ventilation), the cooling results in respiratory depression, or when combined with vagal and carotid chemoreceptor denervation in respiratory arrest (e.g. SCHLAEFKE, 1981). The intensity of ventilation and CO2-H+ responsiveness inhibition raises the assumption that other respiratory activities may be relayed in the I areas on the way to the CRC, and that breathing cannot continue on as usual when the I areas are shut down. A few reports failed, however, to substantiate the crucial role of the VMS in the chemoceptive function (COZINE and NGAI, 1967; LIPSCOMB and BOYARSKY, 1972; CRAGG et al., 1977; MALCOLM et al., 1980).

We hypothesized that the inhibitory effects ascribed specifically to blockade of
the I areas may rather depend on the decline in the combined power of reflux inputs reaching and influencing the CRC. The open loop condition could then give a misleadingly exaggerated impression of the respiratory significance of the I areas, since it disrupts a number of reflux rib cage proprioceptive mechanisms. In support of this hypothesis we report that a graded reduction of basic respiratory inputs during a cold block of the I areas may not end in respiratory arrest in anesthetized but otherwise intact cats.

The study was performed on 6 spontaneously breathing cats of 2.8–3.5 kg. The animals were anesthetized with sodium pentobarbital (30 mg·kg⁻¹, i.p.; plus supplementary i.v. doses as needed), tracheostomized, and subjected to the surgical procedure of exposing the VMS. The detailed procedure and topography of the chemoeceptive areas are described elsewhere (Pokorski, 1976). The central respiratory output was monitored from the integrated efferent C₃ phrenic nerve activity according to the method of Huszczuk and Widdicombe (1973). Minute respiratory output representing an index of pulmonary ventilation was computed as a product of peak tidal phrenic amplitude (AT) and breath frequency (f). Along with the phrenic activity, thermode temperature, end-tidal CO₂ tension (PAco₂), and arterial blood pressure (AP) were monitored continuously.

Bilateral cooling of the I areas was achieved by a two-footed copper thermode, through which ethyl chloride flowed continuously as a cooling medium. Tap water of preadjusted temperature was used for flushing and rewarming. Temperature of the thermode was monitored with a thermocouple welded into the underside of one of its cooling feet, which were 2 × 3 mm each, the latter being the longitudinal distance. The feet were placed rostromedially to the XIIth cranial nerve. They covered two symmetric areas extending caudally from anterior inferior cerebellar artery to the level of the upper XIIth nerve rootlets, and laterally from about 2.5 mm of the midline. The area covered corresponds roughly to that delineated by Schlaefke and Loeschcke (1967) as the intermediate area. Cooling temperatures in 6 experiments were 9, 10.5, 12, 13, 13, and 14°C each. These temperatures were chosen to approximately match those used in the majority of studies (see e.g. Schlaefke, 1981; Bruce, 1986). According to a study by Benita and Condé (1972) on the spread of cold in the brain stem, this range of cooling temperature should chiefly affect the synaptic function with minimal disturbance to conduction along fibers. The cooling temperatures were achieved rapidly. It took, on average, 16 ± 2 s to achieve a given steady level of temperature. Once it was asserted that prolonged cooling of the I areas had no detrimental effect on the animal’s state, the duration of cooling was extended to up to 20 min. As control, the feet were placed over the pyramids medially to the areas described above. Cooling the pyramids yielded no changes in respiration. The cerebrospinal fluid (CSF) production and flow were unrestricted. Sampling of the CSF pool in consecutive phases of the experiment showed its stable acid-base status. Arterial blood gas content was also checked at frequent intervals (Radiometer BMS3 assembly). The rectal temperature was maintained at 38°C.

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The protocol called for studying the steady-state respiratory responses to cooling the I areas, and to subsequent vagotomy and switching from breathing room air to 100% oxygen executed during the cooling period. In two instances, a switch to hyperoxia was performed after rewarming the I areas, which were then cooled down to the same level. The data for both hyperoxic procedures (hyperoxia during cooling and cooling during hyperoxia), qualitatively the same, were analyzed together. The steady-state data were collected 3–5 min from onset of each procedure. Transient changes occurring in the latter half of the first minute were also quantified. At each point of data collection 8–20 neurograms (their frequency being equal to breathing frequency and peak amplitude proportionate to tidal volume), which included complete breath cycles, were analyzed and averaged per min. The phrenic nerve measurements were normalized to allow comparisons among cats (WALDROP et al., 1982). In each cat, a value of 100 U was ascribed to the highest integrated phrenic activity recorded, which usually was a sigh, and all others were scaled down in relation to that activity. Statistical comparisons between the experimental conditions were made with a one-way analysis of variance; p < 0.05 was considered significant.

The steady-state mean respiratory data in each experimental condition are set out in Table 1. Cooling the I areas increased minute respiratory output significantly. Vagotomy and hyperoxia caused a marked reduction of this output below the precooling level. The respiratory output changes were due chiefly to changes in frequency. The corresponding changes in $P_{\text{ACO}_2}$ reflected the function of intact chemostatic feedback.

Figure 1 shows the steady-state as opposed to transient changes in respiratory output in each condition. The transient changes conformed to the results in the open loop condition (e.g. CHERNIACK et al., 1979; SCHLAEFKE et al., 1979) in that the cooling of the VMS effectively depressed respiratory output. The depression in this

| Table 1. Steady-state mean values of the respiratory indices and blood pressure in the control condition, in response to cooling the ventral medullary surface, to vagotomy and switching to hyperoxia performed during the cooling period (see text for abbreviations). |
|-----------------|----------------|-----------------|-----------------|-----------------|
|                 | Control        | Cooling         | Vagotomy        | Hyperoxia       |
| Resp. out. (U·min$^{-1}$) | 1,322 ± 227 | 2,185 ± 394*   | 707 ± 173**    | 370 ± 73**      |
| $A_t$ (U)       | 37.7 ± 5.3    | 34.9 ± 4.0     | 38.7 ± 12.8    | 26.7 ± 7.9      |
| f (min$^{-1}$)  | 34.4 ± 2.2    | 61.9 ± 5.4*    | 19.7 ± 3.3**   | 14.8 ± 2.6**    |
| $P_{\text{ACO}_2}$ (Torr) | 40.0 ± 1.2 | 40.0 ± 1.3*    | 44.3 ± 1.5**   | 60.3 ± 1.5†     |
| AP (Torr)       | 146 ± 11      | 125 ± 11       | 130 ± 22       | 123 ± 21        |
| n               | 6             | 6              | 4              | 4               |

Values are mean ± S.E.M. *p < 0.05 compared with control. **p < 0.05 compared with both cooling and control. †p < 0.05 compared with both vagotomy, cooling, and control.
study may have been less due to the countereffect of accumulating asphyxic stimulus (hypoxia plus hypercapnia) and of preserved rib cage respiratory afferentation. The transient depression was exacerbated by consecutive procedures up to a fleeting apnea when vagi were cut and carotid chemoreceptors attenuated during cooling.

Following initial depression, an increase in respiratory output occurred in the steady-state of each experimental condition. The asphyxic stimulus likely has played a role in this increase. However, no apparent correlation was found between the increasing asphyxic stimulus in consecutive transient phases of the experiment and the following steady-state respiratory output increase.

The respiratory output increase over the control level in response to cooling alone is in line with the findings of Bruce (1986) who demonstrated tachypnea of lower phrenic amplitude during cooling the I areas to 10°C in kittens. This increase is intriguingly at variance with the findings of another close loop study of Schlaefke and Loeschcke (1967). The discrepancy is not easily explained. In that study tidal volume was affected in contrast to frequency in this study. Thus, a qualitatively different mechanism of respiratory changes is likely. The respiratory depression reported by Schlaefke and Loeschcke (1967) bears similarities to the transient response in this study with respect to the span of the cooling time and

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higher share of tidal volume in respiratory changes. Another issue that can be raised is that our thermode covered a larger surface stretching toward the upper part of the caudal chemoceptive area, and a partial impingement on this area cannot be entirely ruled out. Cooling the caudal area has been shown to strikingly affect frequency (Cherniack et al., 1979).

The mechanism of the respiratory increase in the present study is open to conjecture, but it might depend, besides the effect of asphyxia, on the amplified function of other respiratory inputs preserved, or on gradual spreading of low temperature into deeper structures over time. It is also possible that our results reflect artifact introduced by uneven distribution of temperature change under the feet of the thermode. That could render neural elements partly undercooled and allow some inputs to traverse the areas in question and to retain feedback loops, for which we were unable to control.

A few other technical comments are in order before interpreting these data. Uncertainties often raised concerning the method of cooling of brain surface apply to this study. Deeper neuronal structures may be variably affected depending on thermal conductivity, blood flow, and cooling time. Cooling may also interfere with local biochemical and acid-base processes (Reeves, 1977). Cooling of the blood in rich superficial network of vessels is noted as a potential modifier of deeper structures' function (Lipscomb and Boyarsky, 1972). For all its ambiguities, the method of cooling is thought to affect a fairly restricted space. Schlaefke and Loeschcke (1967) showed that when the thermode tip was at 9°C, tissue 1 mm apart was at 34°C. Benita and Condé (1972) showed that the 37°C isotherm for cooling to 5°C was 2 mm distant from the cryogenic probe. Similar findings were also reported by Brooks et al. (1973). These findings may not be fully applicable to prolonged cooling, which might be expected to affect distant tissue more severely.

The present study differs from most others in two respects: the longlasting cooling and the close loop condition in spontaneously breathing animals. The study demonstrates differences between the short- and long-term effects of cooling the I areas. If the steady-state respiratory output increase were due to the accumulated blood chemical stimuli, the existence of other than central, eliminated by cooling, or peripheral, attenuated by hyperoxia, chemoceptive mechanisms should be considered. Several other possible mechanisms unrelated to known chemoception may be conceived. Asphyxia leads to increased H⁺ concentration. That may produce a general excitatory state and in this way stimulate respiration or have a direct but nonspecific stimulatory effect on the dorsal respiratory group of neurons, bypassing the VMS. The proprioceptive innervation of the rib cage (Corda et al., 1965) and nonrespiratory activities of the sinus nerve (Schlaefke et al., 1979) may be mentioned as possible sources of enhanced respiratory output through spinal reflexes and ascending information to supraspinal levels.

While this study has not identified the determinants of the secondary respiratory output increase in prolonged cooling of the I areas, it calls into question the pivotal role frequently ascribed to these areas in respiratory regulation in the
lose loop condition. It appears as if the VMS structures have constituted just one of the many inputs to the CRC. This input may be interfered with by thermal and likely other factors. The CRC may adjust its state according to the summed afferent inputs it receives. The cold block of the I areas appears to leave the storage and memory capabilities of the CRC intact, so that its neuronal network can sort out incomplete inputs and restore the missing information, leading to respiration increase after the initial depression.

To sum up, I areas blockade did not hamper the respiratory output appreciably as long as other inputs to the CRC were maintained operational. Further experiments are required assessing the effect of muscle paralysis and disruption of reflux respiratory proprioception on the responses to cooling the I areas to establish the proposed mechanisms.

We thank Drs. Y. Honda and Y. Fukuda for helpful critical comments on the manuscript. This study was supported in part by the CPBP Grant No. 06-02.

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