Cholinergic Mechanism in the Cerebrovascular Action of Carbon Dioxide

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Abstract: Cholinergic Mechanism in the Cerebrovascular Action of Carbon Dioxide

The effect of an increase in $P_{a\text{CO}_2}$ on cortical blood flow was tested in control animals and after atropine or eserine administration. Blood flow was measured at the tip of implanted platinum electrodes by means of the hydrogen clearance method. The results showed that, although atropine did not impede the appearance of autoregulation, it completely blocked the increase in cortical blood flow associated to a step increase in $P_{a\text{CO}_2}$. The effect of CO$_2$ on cortical blood flow was significantly greater under eserine than in untreated controls.

Additional Key Words atropine eserine $CO_2$ cholinergic cortical blood flow

Introduction

The relationship between $P_{a\text{CO}_2}$ and cerebral blood flow (CBF) is a well-known fact. It has been shown that inhalation of CO$_2$ induces an increase in CBF. More precisely, CBF and pial precapillary vessels' diameter have been found to be a continuous function of arterial $P_{\text{CO}_2}$. It is currently accepted that the dilator action of CO$_2$ on cerebral vessels is the result of changes in extracellular pH or $P_{\text{CO}_2}$ that would in turn act on smooth muscle cells. An alternative explanation, however, is that CO$_2$ might excite nerve cells of a neurogenic vasodilatory system. Experiments showing a blockade of the vasodilatory action of CO$_2$ after localized lesions of the brain stem or in cerebro isolée preparations give support to the latter interpretation. As an increase in $P_{a\text{CO}_2}$ is associated with acetylcholine release at the cerebral cortex and there are evidences for the existence of cholinergic dilatory receptors on cortical vessels, it was considered of interest to test the effect of drugs affecting cholinergic transmission on the vasodilatation induced by CO$_2$.

In a previous paper we found that the increase in cortical blood flow that accompanies desynchronization is mediated, at least in part, by a cholinergic mechanism and, as an increase in $P_{a\text{CO}_2}$ can induce cortical desynchronization, the experiments were always performed at a level of urethane anesthesia such that inhalation of CO$_2$ never led to cortical desynchronization.

Methods

Male albino rats were used. The animals were anesthetized with urethane intraperitoneally 1.5 gm per kilogram, tracheostomized, and fixed to a nose clamp. The frontoparietal cortex was exposed and a bare platinized wire (electrodes), 30 μ in diameter, was inserted 1 mm into the cortex. The electrodes were inserted to record spontaneous electrical activity and local blood flow from hydrogen desaturation slopes as described elsewhere. The cortex was exposed and the insertion visually controlled by means of a stereoscopic microscope and the tip displacement measured in the dial gauge of the micromanipulator. As an increase in $P_{a\text{CO}_2}$ is associated with acetylcholine release at the cerebral cortex and there are evidences for the existence of cholinergic dilatory receptors on cortical vessels, it was considered of interest to test the effect of drugs affecting cholinergic transmission on the vasodilatation induced by CO$_2$.

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urethane (Merck), atropine sulphate (Purest), and eserine sulphate (Sandoz). Artificial CSF was prepared according to Merlis.11

**Results**

**EFFECT OF INTRAPERITONEAL ATROPINE**

In a group of animals CBF was measured when they were breathing air or a mixture of CO₂ in air. In a second group CBF was measured in the same conditions but after an intraperitoneal injection of 1 mg of atropine sulphate. Mean Pa₉0₂ when animals were breathing the CO₂-air mixture was 55.4 ± 2.82 and Pa₉0₂ when they were breathing air was 40.9 ± 1.58. The results showed that atropine completely blocked the increase in CBF induced by CO₂ (fig. 1) although it did not affect the basal CBF when animals were breathing air (fig. 2). The values of mean arterial pressure when animals were breathing air or CO₂ in air were compared both without treatment and under atropine (table 1). Inhalation of CO₂ induced a nonsignificant increase in blood pressure in untreated animals, but under atropine a significant decrease occurred. In view of these facts the state of cerebrovascular autoregulation was studied by plotting cerebrovascular resistance (CVR) as a function of mean arterial pressure (MAP) in all the experimental conditions. As can be seen in figure 3a, inhalation of CO₂ did not impair autoregulation as CVR is still linearly correlated to MAP although the slopes are significantly different (cortical blood flow is autoregulated but at a higher level). Under atropine (fig. 3b) CVR and MAP were linearly correlated in both conditions but the slopes of CVR as a function of MAP breathing air or CO₂ in air did not change.

**EFFECT OF INTRAPERITONEAL ESERINE**

In a second series of experiments, the ability of a cholinesterase inhibitor to potentiate the cerebrovascular action of CO₂ was tested. Once the cerebrovascular effect of CO₂ was established, the
CHOLINERGIC MECHANISM IN CO₂ ACTION

TABLE 1
Mean Arterial (Femoral) Pressure in the Different Experimental Conditions (mm Hg; Mean ± SE)

<table>
<thead>
<tr>
<th></th>
<th>No treatment</th>
<th>Atropine</th>
<th>P of the difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>46.56 ± 3.49</td>
<td>58.06 ± 3.74</td>
<td>0.005</td>
</tr>
<tr>
<td>CO₂ in air</td>
<td>53.21 ± 4.28</td>
<td>43.02 ± 3.15</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

animal received 75 μg of eserine sulphate intraperitoneally, after which the effect of CO₂ on CBF was again tested. As can be seen in figure 1, under eserine, the change in CBF induced by CO₂ was considerably increased.

Discussion
The decrease in cerebrovascular resistance induced by an increase in PaCO₂, that is confirmed in the present experiments, has been currently ascribed to direct action of CO₂ on cerebrovascular smooth muscle. The results reported here seem to argue against this interpretation, as the effect is blocked in the atropinized animals. It is suggested that the effect of CO₂ on cerebrovascular resistance is exerted, at least in the present experimental conditions, through a mechanism involving a neuronal cholinergic step, as it can be blocked by atropine and potentiated by eserine. (It is interesting to note that CBF does not change when atropine is given to an animal breathing air.) Furthermore, even under atropine, CBF is autoregulated with respect to blood pressure, that is, CVR is a linear function of mean blood pressure. Atropine, however, impedes the decrease in slope of that function brought about in the control condition by the elevation of PaCO₂. The latter implies the existence of two different mechanisms for the autoregulation of cortical blood flow and the cerebrovascular action of CO₂, as one remains unaffected and the other is blocked by atropine. The interpretation could be that the PaCO₂ sets the level of CBF autoregulation through a neurogenic mechanism with a cholinergic step, being autoregulation itself supported by a noncholinergic mechanism.
Other authors\(^{12, 13}\) have reported loss of autoregulation in hypercapnia, probably due to a maximum vasodilation. In our experiments, in which hypercapnia was less severe, autoregulation remained intact although the vasodilatory effect of CO\(_2\) was clearly observed.

A direct effect of CO\(_2\) on cortical vascular smooth muscle cannot be discarded from our results, however, since we have not measured the actual change in cortical P\(_{CO_2}\) or pH at the level of pial vessels in our experiments. These would not necessarily follow the changes in P\(_{aCO_2}\) as the cortex is exposed with the purpose of recording.

**References**

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