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Cerebrovascular Response to CO_2 Inhalation in Unanesthetized Goats (40401)

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Introduction. Attempts to relate the cerebral vasodilatation resulting from high CO_2 ventilation to neurogenic influences have been inconclusive. A number of studies in man (1–3) and in anesthetized baboons (4) have shown that α or β adrenergic receptors play little or no role in the response of cerebral vessels to CO_2 inhalation. Also, results from human and animal studies did not clarify whether an alpha adrenergic mechanism participates in the cerebral vasoconstriction observed during hypocapnia (5–8). The finding that atropine or section of the VIIth cranial nerve partially blocks the cerebral vasodilatation produced by CO_2 in anesthetized animals (9–12) would suggest that cholinergic mechanisms are somehow implicated in the cerebrovascular sensitivity to CO_2; these findings have also been questioned recently (13).

It was the objective of the present study to evaluate, in the unanesthetized goat, the effects on the cerebral circulation of CO_2 inhalation, before and after selective, localized blockade of the adrenergic and cholinergic receptors present in cerebral blood vessels.

Methods. Thirteen female goats weighing between 35–50 kb were used. In this species each internal maxillary artery provides the total blood flow to each cerebral hemisphere via the rete mirabile; the vertebral arteries do not contribute to brain blood flow and the extracranial internal carotid artery is absent (14–16). The operative procedure to measure blood flow has been described in detail before (16). Briefly, under 2% sodium thiopental anesthesia, the extracerebral vessels of one of the internal maxillary arteries were ligated and thrombosed with 1000 NIH units of thrombin (thrombin, topical, Parke, Davis and Co.) dissolved in 0.5 ml of saline. This maneuver produces an almost immediate obliteration of the ethmoidal, ophthalmic, and buccinator arteries, thus eliminating blood flow to the eye and other facial tissues. This is confirmed upon recovery from surgery by the production of ipsilateral blindness; there is apparently enough collateral circulation supplying facial structures to prevent necrosis. An electromagnetic flow transducer (Biotronex) was placed on the internal maxillary artery to measure blood flow to the ipsilateral cerebral hemisphere. A polyethylene catheter was inserted in the temporal artery and permanently fixed to permit injection or infusion of drugs directly into the internal maxillary artery; the same catheter was used to measure arterial blood pressure with a Statham transducer. A snare-type occluder was placed on the external carotid artery to obtain zero flow baselines. The external connecting leads from the flow transducer and occluder and the temporal artery catheter were led out subcutaneously and secured to the goat's horn.

Heart rate was measured from the arterial pressure pulse with a rate meter. Flow measurements were made with a Biotronex electromagnetic flowmeter (model BL-610). Cerebral blood flow, arterial blood pressure, and heart rate were recorded on a Beckman recorder. The experiments on the unanesthetized goat started 2–3 days after the operative procedure, at which time the goat had fully recovered and in a steady cardiorespiratory state.

The goats breathed room air through a mask attached to a two-way valve with a minimum dead space. When all measurements had been constant for 15 min, the mask was abruptly connected to a bag containing 9% CO_2 in air for a period of 10–20 min. This procedure was repeated after selective blockade of the adrenergic (α or β) or cholinergic atropine-sensitive vascular receptors. Cerebral blood flow, arterial blood pressure, and heart rate were continuously recorded, first during air breathing and then during inhalation of the gas mixture.

Without previous training the goats became, in general, readily accustomed to the breathing mask and the sensations of carbon

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dioxide inhalation. Those animals which showed signs of excitation or discomfort shortly after CO_2 respiration started, were not utilized in this study.

Alpha adrenergic blockade of the cerebral circulation was produced in four goats by slow infusion of phentolamine (Regitin, Ciba) into the internal maxillary artery (1–1.5 mg in 1 ml of saline over a period of 10–15 min); propranolol (1 mg, Sumial ICI Farma) was used in a similar manner in three goats; and atropine (1–2 mg, Atropina Miró) was used in three goats. The remaining three animals were subjected to alternate adrenergic and cholinergic blockade. In the latter three goats a period of at least 24 hr was allowed between adrenergic (α or β) and cholinergic blockade.

The dose of phentolamine used effectively blocks the cerebral vasoconstriction produced by norepinephrine (17) or cervical sympathetic nerve stimulation (18) whereas propranolol and atropine significantly decrease the vasodilatation which follows the administration of isoproterenol (18) and acetylcholine (19), respectively.

Arterial blood was analyzed before and during carbon dioxide inhalation for pH, P_{CO_2}, and P_{O_2} by standard electrometric methods (Corning Scientific Instruments, model 165, Medfield, Mass.).

The statistical analysis was done by means of the Student's t test; a probability value of less than 5% was considered significant ($P < 0.05$).

Results. The effects of CO_2 inhalation on cerebral blood flow, systemic arterial blood pressure, and heart rate are illustrated in Fig. 1 and the average data from all the experiments are summarized in Table I. Along with the increased cerebral blood flow secondary to CO_2 inhalation ($P < 0.05$) there was a small but significant rise in mean arterial blood pressure ($P < 0.05$). Arterial P_{CO_2}, P_{O_2}, and hydrogen ion concentration all increased as expected (Table II).

Administration of 1–1.5 mg of phentolamine directly into the internal maxillary artery produced an increase in resting cerebral blood flow of 30% on the average without altering systemic arterial blood pressure or heart rate. Inhalation of CO_2 under these conditions increased cerebral blood flow to a level which did not differ significantly ($P > 0.05$) from that found in the untreated state (Fig. 2 and Table I). Blockade of beta adrenergic receptors with propranolol (1–2 mg) reduced resting cerebral blood flow by 12% but the cerebrovascular response to hypercapnia was not significantly different ($P > 0.05$) from that obtained before treatment (Fig. 3 and Table I). Administration of atropine in doses of 1–2 mg caused no significant alterations ($P > 0.05$) of resting cerebral blood flow and did not appreciably affect the increase in cerebral blood flow induced by CO_2 ($P > 0.05$, Fig. 4 and Table I).

Discussion. The present experiments show that high CO_2 inhalation produces blood gas alterations and cerebral blood flow changes in the goat similar to those reported in normal unanesthetized men (20, 21).

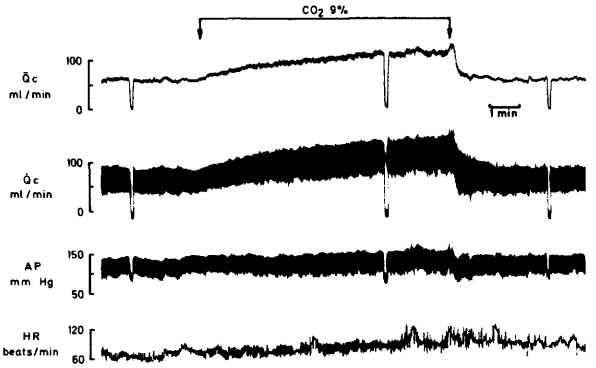

FIG. 1. Representative tracings of mean ($\bar{Q}c$) and pulsatile ($\dot{Q}c$) left hemispheric cerebral blood flow, systemic arterial blood pressure (AP), and heart rate (HR) before, during, and after 9% CO_2 inhalation in unanesthetized goat. Deflections of $\bar{Q}c$, $\dot{Q}c$, and AP records correspond to occlusion of external carotid artery to obtain zero flow.

TABLE I. EFFECTS OF CO_2 INHALATION IN UNANESTHETIZED GOATS.[a]

	Control ($n = 13$)		With phentolamine ($n = 7$)		With propranolol ($n = 6$)		With atropine ($n = 6$)	
	Air	CO_2	Air	CO_2	Air	CO_2	Air	CO_2
CBF ml/min per 100 g	118 ±8.36	185 ±15.52	153 ±12.18	219 ±18.56	103 ±11.20	179 ±17.68	107 ±11.10	183 ±21.36
MAP mmHg	106 ±1.64	114 ±1.66	102 ±2.00	107 ±1.80	100 ±2.10	108 ±1.76	106 ±2.20	117 ±1.90
HR beats/min	73 ±3.04	78 ±2.32	81 ±3.0	83 ±2.6	70 ±1.7	72 ±2.0	92 ±3.12	90 ±2.83

[a] n is number of goats. Values are means ± SEM. CBF = cerebral blood flow. MAP = mean arterial blood pressure. HR = heart rate.

TABLE II. ARTERIAL BLOOD GASES BEFORE AND DURING EFFECTS OF CO_2 INHALATION.[a]

	Air	CO_2
pH	7.41 ± 0.01	7.26 ± 0.01*
P_{O_2}	78 ± 1.80	97 ± 2.41*
P_{CO_2}	33 ± 1.32	50 ± 0.94*

[a] Values are means ± SEM obtained from 13 goats. * = Statistical significance ($P < 0.05$).

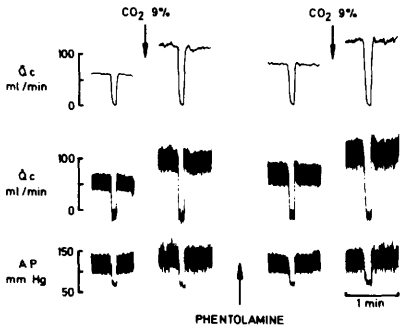

FIG. 2. Sections of an experimental record showing the effects of CO_2 inhalation on cerebral blood flow and arterial blood pressure before and after administration of phentolamine into the internal maxillary artery. Symbols as in Fig. 1.

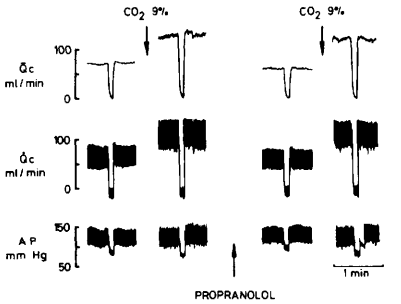

FIG. 3. Sections of an experimental record showing the effects of CO_2 inhalation before and after administration of propranolol. Symbols as in Fig. 1.

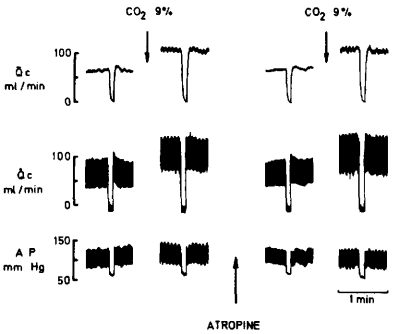

FIG. 4. Sections of an experimental record showing the effects of CO_2 inhalation before and after administration of atropine into the internal maxillary artery. Symbols as in Fig. 1.

The administration of 1–1.5 mg of phentolamine into the internal maxillary artery produced a 30% increase in cerebral blood flow without altering systemic arterial blood pressure or heart rate. This increase in cerebral blood flow is due to the loss of an existing alpha adrenergic tone in the cerebral blood vessels since phentolamine fails to produce cerebral vasodilatation after depletion of catecholamine stores with reserpine (18) or after removal of both superior cervical sympathetic ganglia (22). Our data show that under conditions of alpha blockade of the cerebral vessels, CO_2 inhalation produced increases in cerebral blood flow similar to those obtained before phentolamine. Moreover, the net increase in cerebral blood flow produced by CO_2 was similar in control and phentolamine-treated animals. Thus it seems reasonable to suggest that the alpha adrenergic receptors are not involved in the cerebral vasodilatation produced in hypercapnia. These results are in agreement with previous reports

showing that the cerebral blood flow response to CO_2 in man (1, 3) and anesthetized baboons (4) is not modified after administration of α blockers.

Contrary to the effects of phentolamine, propranolol decreases cerebral blood flow probably due to the loss of a tonic vasodilatatory component exerted in normal conditions by the α adrenergic receptors. In relation to this it has recently been shown that the small cerebral vasoconstrictor response to propranolol disappears after removal of the superior cervical sympathetic ganglia (22). In addition to the blocking effects on cerebral vascular receptors it has been suggested that part of the reduction of cerebral blood flow induced by propranolol might be secondary to changes in cerebral metabolism (2, 23, 24). With regard to the possible excitation of beta adrenergic receptors during CO_2 inhalation our data show that propranolol does not influence the cerebral vasodilatation induced by respiratory acidosis. These results confirm studies in patients with stroke showing that cerebral vessels reactivity to changes in Pco_2 is unchanged by beta blockade (2); however, they differ from those reported by Aoyagi *et al.* (25) who observed in anesthetized baboons a decrease in cerebral sensitivity to CO_2 after intravertebral infusion of propranolol.

Our results in unanesthetized goats do not permit a conclusion as to the existence of a cholinergic vasodilator tone in cerebral vessels since atropine did not significantly change resting cerebral blood flow. This is in agreement with the observations where various experimental approaches were used showing that the parasympathetic tone to the cerebral vessels is probably irrelevant (9, 13, 26, 27). Blockade of the cholinergic atropine-sensitive receptors existing in the cerebral blood vessels did not change the response to CO_2 inhalation in any of the six animals shown in Table I. This indicates that the cerebral vasodilatation induced by CO_2 does not result from stimulation of these receptors. Previous studies in anesthetized baboons have shown that injections of atropine into the vertebral artery decrease cerebral vasomotor responses to changes in Pco_2 suggesting the existence of a central cholinergic system located presumably in the brain stem and diencephalon-innervating cerebral vessels (9). Other workers have made the same proposal based on experiments in anesthetized rats showing that intraperitoneal injections of atropine blocked the increase in cortical blood flow associated to hypercapnia (11). In agreement with our results it is interesting to note that unilateral or bilateral section of the VIIth cranial nerve, which apparently carries the parasympathetic fibers innervating cerebral vessels, does not alter the response to hypercapnia (13).

It is concluded that adrenergic blockade of cerebral blood vessels produces changes in cerebral blood flow but does not alter the cerebral vasodilatation induced by CO_2 inhalation. Furthermore, the present data suggest that blockade of the cholinergic atropine-sensitive receptors existing in the vessel wall has no effects on the cerebral vasomotor response to hypercapnia.

Summary. The effects of cerebral blood flow of inhalation of 9% CO_2 in air were examined in unanesthetized goats. This procedure increased mean cerebral blood flow from 118 to 185 ml/min per 100 g and was accompanied by a rise in Pco_2 from 33 to 50 mmHg and a fall in pH from 7.41 to 7.26. Administration of phentolamine, propranolol, or atropine into the internal maxillary artery did not modify the normal cerebrovascular response to CO_2 inhalation. It is concluded that blockade of vascular adrenergic of cholinergic atropine-sensitive receptors in the goat has no effect on the cerebral vasomotor response to hypercapnia.

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