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The Effects of Carbon Dioxide on Preganglionic Sympathetic Activity during Halothane, Methoxyflurane, and Cyclopropane Anesthesia

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The effects of 2.5 and 5.0 per cent inspired CO_2 on cervical preganglionic sympathetic activity during exposure to equipotent concentrations of halothane, methoxyflurane, and cyclopropane have been compared with values during basal anesthesia with nitrous oxide–oxygen and gallamine in six cats. Although none of the three anesthetics changed the *fractional increase* in sympathetic nervous activity produced by CO_2 from that observed during basal anesthesia, halothane and methoxyflurane depressed the *absolute increase*, while cyclopropane had no effect. (Key words: Carbon dioxide; Preganglionic sympathetic nervous system; Halothane; Methoxyflurane; Cyclopropane.)

GENERAL ANESTHESIA (cyclopropane, halothane, and methoxyflurane) has been claimed to modify the circulatory as well as the sympathoadrenal responses to respiratory acidosis. Halothane was found to reduce the tachycardia, hypertension, and increase in plasma norepinephrine concentration produced by carbon dioxide in awake man, while cyclopropane enhanced the increase in plasma catecholamine concentrations even though it reduced both the attendant tachycardia and the hypertension.[1]

Animal studies have shown that plasma catecholamine concentrations increase with respiratory acidosis during both halothane[2] and methoxyflurane[3] anesthesia. No study directly measuring changes in sympathetic nervous activity induced by CO_2 during anesthesia with equipotent concentrations of cyclopropane, halothane, and methoxyflurane has yet been carried out, and we report below an attempt to do so, using the data of Brown and Crout[4] to define equipotent anesthetic concentrations in cats.

Methods

Subjects of the study were six cats weighing 1.7 to 3.0 kg. They were initially anesthetized with halothane in O_2 while the trachea, a femoral artery, and a vein were cannulated. Halothane was then discontinued, and gallamine, 20 mg, was given intravenously at once and at half-hourly intervals thereafter throughout the study. Respiration was controlled with a Harvard animal pump unless cyclopropane was in use, when a Bird respiratory with a Frumin valve at the connection of the tracheal cannula was employed. Regardless of which respirator was used, the outflow from the anesthesia machine was 2 l nitrous oxide and 2 l oxygen per minute. A 5-l reservoir bag with a pop-off valve was interposed in front of the Harvard respirator to prevent back-pressure on the anesthesia machine and to permit escape of excess gas.

The esophagus and trachea were divided low in the neck, tied over a metal rod, and the pharynx and larynx pulled out through the mouth to give access to the cervical sympathetic trunks. The left trunk was divided just below the superior cervical ganglion, dissected free of surrounding tissue, placed on a metal backplate and immersed in mineral oil. Multi-fiber strands were teased free and placed on bipolar platinum wire electrodes connected to a Grass P-5 amplifier. From here the output went to a Grass audio-amplifier and to one channel of a Tektronix 565 oscilloscope. The signal from a vertical oscilloscope amplifier was fed to a pulse-height selector comprising both discriminator and pulse-shaping circuits, so that action potentials between upper

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and lower voltage limits could be selected, converted into square-wave pulses, and counted on a Nuclear Chicago ratemeter (calibrated over the range 0–300 cps) and displayed on a Grass multichannel recorder. The output of the pulse-height selector was monitored continuously to ensure that a change in the signal-to-noise ratio did not affect the number of action potentials counted during an experimental sequence. This was done by using the pulse-height selector output to trigger a Venner electronics reset unit (TS32), which caused the resetting to zero of a time scale which was displayed on the oscilloscope. Audiomonitoring of the sympathetic discharge was also used continuously throughout every experiment.

To insure that we were recording from sympathetic fibers, an intravenous injection of epinephrine (5 μg) was given initially and only fibers showing moderate inhibition of activity during the increase in blood pressure were studied further. Unresponsive fibers were discarded and new ones sought. The arterial pressure was sensed by a Statham P-23D transducer and recorded on a Grass recorder. Mean pressures were obtained by electrical damping. Arterial blood samples (3 ml) were drawn at 90–120-minute intervals and analyzed in an I.L. assembly for *p*H, P_{CO_2} and P_{O_2}. Blood withdrawn was replaced with an equal volume of physiologic saline solution. The measured Pa_{O_2} exceeded 100 mm Hg in every sample. Metabolic acidosis, when present, was corrected with $NaHCO_3$ on the basis of base deficit × kg body weight × 0.3 mEq. Rectal temperature was measured with a Yellow Springs thermistor and maintained at 37–38 C with the aid of a "K"-pad (Gorman-Rupp). Halothane was delivered from a calibrated Fluotec vaporizer, methoxyflurane from a calibrated Foregger Pentomatic vaporizer.

CO_2 was administered by replacing the normal oxygen supply to the anesthesia machine with a tank containing 5 per cent CO_2 in O_2 or one containing 10 per cent CO_2 in O_2. These mixtures were diluted with the same volume of either nitrous oxide or nitrous oxide and cyclopropane to give inspired carbon dioxide concentrations of 2.5 or 5.0 per cent. End-expired CO_2 was measured continuously by a Godart capnograph and kept as close as possible to 5.4 per cent in the periods between CO_2 administration. Corrections were made for the spectral absorption caused by nitrous oxide and cyclopropane. End-expired concentrations of halothane and methoxyflurane were measured by gas chromatography (Hewlett Packard #700), the samples being withdrawn in 5-ml increments into a 50-ml syringe. Cyclopropane was measured by the method of Linde and Price.[5]

Experimental Protocol

Before the administration of any but the basal anesthetic, 2.5 per cent CO_2 was administered for 5 minutes (counted from the time an increase in inspired CO_2 was noted on the capnograph). The sympathetic activity (SA) in impulses/second and mean arterial pressure (MABP) during the fifth minute were recorded. The inspired CO_2 concentration was then raised to 5 per cent and measurements made as before at the fifth minute. CO_2 was then discontinued. Such a sequence of CO_2 administration will hereafter be referred to as a "CO_2-stimulation trial." The end-expired CO_2 concentration achieved in this initial trial was duplicated during the rest of the study whenever CO_2 was administered, if necessary by changing the rate of the respirator. The anesthetics were administered in randomized order but in equipotent doses at the time of CO_2 stimulation. The minimal alveolar anesthetic concentrations (1 MAC) were[4]: halothane 0.82 ± 0.1 per cent, methoxyflurane 0.23 ± 0.002 per cent, and cyclopropane 19.7 ± 0.95 per cent. We gave each agent in a concentration (end-expired) equivalent to 1.5 MAC. (We are aware that with cyclopropane the additive anesthetic effect from the N_2O is slightly less than with the other two agents.)

Halothane was administered at a 3 per cent concentration for 2 minutes, 2 per cent for 2 minutes, and then continued with 1.5 per cent to a total exposure time of 15 minutes. MABP, SA, and end-expired halothane concentrations were then measured, a CO_2-stimulation trial carried out, end-expired halothane again measured, and halothane as well as CO_2 discontinued.

Methoxyflurane was administered in a 1 per cent inspired concentration for 15 minutes,

TABLE 1. The Effects of Halothane, Methoxyflurane and Cyclopropane on Sympathetic Nervous Activity and Mean Arterial Blood Pressure*

	Control	Halothane	Control	Methoxy-flurane	Control	Cyclo-propane
Sympathetic frequency (imps/sec)						
Cat 1 (H,M,C)	44	40	80	80	100	110
Cat 2 (M,H,C)	90	50	80	110	85	88
Cat 3 (C,H,M)	20	9	30	20	60	60
Cat 4 (C,M,H)	120	80	105	90	140	120
Cat 5 (H,C,M)	40	28	70	32	90	120
Cat 6 (M,C,H)	45	17	60	45	55	65
MEAN	59.8	37.3†	70.8	62.8	88.3	93.8
SE	15.3	10.5	10.2	14.6	12.6	11.0
Mean arterial blood pressure (mm Hg)						
Cat 1 (H,M,C)	114	74	134	94	112	75
Cat 2 (M,H,C)	130	78	115	90	130	126
Cat 3 (C,H,M)	122	103	128	111	102	142
Cat 4 (C,M,H)	120	83	142	111	151	151
Cat 5 (H,C,M)	140	102	144	110	153	162
Cat 6 (M,C,H)	106	66	105	76	105	135
MEAN	122.0	84.3§	128.0	98.7§	125.5	131.8
SE	4.9	6.2	6.3	5.9	9.3	12.5

* Control measurements were made before the administration of an anesthetic. The sequences in which the anesthetics (*H*alothane, *M*ethoxyflurane, *C*yclopropane) were administered are indicated in parentheses.
† $P < 0.02$. § $P < 0.01$.

and SA, MABP, and end-expired methoxyflurane concentration measured. The inspired methoxyflurane concentration was then lowered to 0.75 per cent and a CO_2-stimulation trial carried out. End-expired methoxyflurane was then measured again, and the anesthetic as well as CO_2 discontinued.

Cyclopropane was administered in a 30 per cent concentration for 12 minutes before measurements of SA, MABP, and end-expired cyclopropane concentration; a CO_2-stimulation trial was then carried out, end-expired cyclopropane again measured, and the anesthetic and CO_2 discontinued.

Elimination times of 15 minutes for cyclopropane, 20 minutes for halothane, and 30 minutes for methoxyflurane were allowed before a final control CO_2 stimulation trial was carried out (making it the initial control trial for the anesthetic to be administered next).

To compare the effect of CO_2 on the sympathetic frequency with different initial activity levels we have defined the responsiveness to CO_2 as sympathetic frequency during the fifth minute of CO_2 administration divided by sympathetic frequency prior to CO_2. The responsiveness during administration of an anesthetic is then compared with a control responsiveness calculated as the arithmetic mean of the responsiveness to CO_2 found before and after the administration of the anesthetic.‡ Statistical analyses were done using paired t tests.

Results

The concentrations of the three anesthetic agents achieved were (mean ±SE): halothane 1.25 ± 0.05 per cent, methoxyflurane 0.35 ± 0.03 per cent, and cyclopropane 25.8 ± 0.7 per cent—essentially equipotent concentrations. The effects of the anesthetics on sympathetic frequency and mean arterial pressure measured before the start of the CO_2-stimulation trials are shown in table 1. It is evident that halothane significantly ($P < 0.02$) reduced sympathetic activity, whereas neither cyclopropane nor methoxyflurane caused any

‡ Expressed mathematically, the relative response during anesthesia is $= \frac{A}{\frac{1}{2}(C_1 + C_2)}$. Where A = the response *during anesthesia* = SA at 5 minutes CO_2/SA before CO_2 and C_1 and C_2 = the same ratios measured before and after administration of the anesthetic (first and second control responses).

TABLE 2. The Effects of CO_2 on Sympathetic Frequency (imps/sec) during Halothane, Methoxyflurane and Cyclopropane Anesthesia*

	Halothane			Methoxyflurane			Cyclopropane		
	No CO_2 Added	2.5 Per Cent CO_2	5 Per Cent CO_2	No CO_2 Added	2.5 Per Cent CO_2	5 Per Cent CO_2	No CO_2 Added	2.5 Per Cent CO_2	5 Per Cent CO_2
Sympathetic frequency (imps/sec)									
Cat 1 (H,M,C)	40	72	100	80	90	95	110	140	155
Cat 2 (M,H,C)	50	60	70	110	120	125	88	100	120
Cat 3 (C,H,M)	9	11	13	20	35	45	60	95	110
Cat 4 (C,M,H)	80	100	120	90	105	125	120	150	175
Cat 5 (H,C,M)	28	42	64	32	70	110	120	160	210
Cat 6 (M,C,H)	17	19	42	45	125	140	65	110	130
MEAN	37.3	52.3‡	68.2§	62.8	90.8†	106.7†	93.8	125.8§	150.0§
SE	10.5	13.1	15.8	14.6	13.9	13.8	11.0	11.3	15.4
Control mean	70.3	106.2§	130.8§	75.4	114.3§	144.5§	86.8	127.3§	149.5§
Control SE	10.4	10.3	9.6	10.0	12.5	12.4	8.0	9.8	10.8
Arterial pressure (mm/Hg)									
Cat 1 (H,M,C)	74Δ	80Δ	80Δ	94	83	85	79	69	72
Cat 2 (M,H,C)	78	96Δ	90Δ	90	97	100	126Δ	126Δ	122Δ
Cat 3 (C,H,M)	103Δ	100Δ	98Δ	111	124	115	142Δ	136Δ	134Δ
Cat 4 (C,M,H)	83	86	94	111	120	124	151Δ	142Δ	140Δ
Cat 5 (H,C,M)	102	108Δ	105Δ	110	118	121	162Δ	177Δ	169Δ
Cat 6 (M,C,H)	66	82	82	76	87	95	135	124Δ	114Δ
MEAN	84.3	92.0	92.8	98.7	104.8	106.7	131.8	129.0	125.2
SE	6.2	4.5	3.3	5.9	7.4	6.4	12.5	14.3	13.1
Control mean	130.5	138.5‡	139.5	126.7	133.7†	136.3	126.8	133.5§	133.8
Control SE	5.7	4.6	7.0	5.6	6.2	8.2	8.7	8.7	10.0

* Mean values were obtained in the fifth minute of CO_2 administration. Control values are the comparable values obtained with CO_2 administration during basal anesthesia.
† $P < 0.05$. ‡ $P < 0.02$. § $P < 0.01$. Δ Ventricular extrasystoles present.

significant change. Mean arterial pressure was depressed by both halothane ($P < 0.001$) and methoxyflurane ($P < 0.001$), but was essentially unaltered by cyclopropane.

Table 2 shows progressive increases in sympathetic activity with increasing CO_2 concentrations irrespective of the anesthetic agent used. For the response to CO_2 during basal

TABLE 3. Sympathetic Nervous Responsiveness* to CO_2 during Halothane, Methoxyflurane and Cyclopropane Anesthesia

	Control	Halothane		Control	Methoxyflurane		Control	Cyclopropane	
	2.5 Per Cent	5.0 Per Cent	2.5 Per Cent	5.0 Per Cent	2.5 Per Cent	5.0 Per Cent	2.5 Per Cent	5.0 Per Cent	2.5 Per Cent	5.0 Per Cent
Cat 1 (H,M,C)	1.52	2.25	1.80	2.50	1.60	1.97	1.13	1.19	1.41	1.59	1.27	1.41
Cat 2 (M,H,C)	1.17	1.33	1.20	1.40	1.28	1.50	1.09	1.14	1.18	1.37	1.14	1.36
Cat 3 (C,H,M)	1.81	2.49	1.22	1.44	1.93	3.73	1.75	2.25	1.49	1.81	1.58	1.83
Cat 4 (C,M,H)	1.29	1.51	1.25	1.50	1.33	1.56	1.17	1.39	1.33	1.56	1.25	1.46
Cat 5 (H,C,M)	1.78	2.15	1.50	2.29	1.68	2.50	2.19	3.44	1.85	2.44	1.33	1.75
Cat 6 (M,C,H)	2.14	2.52	1.71	2.47	1.74	1.90	2.78	3.11	1.75	1.91	1.69	2.00
MEAN	1.62	2.04	1.45	1.93	1.59	2.19	1.69	2.09	1.50	1.78	1.38	1.64
SE	0.15	0.21	0.11	0.22	0.10	0.34	0.28	0.41	0.10	0.15	0.09	0.11

* As defined in the text. Control is responsiveness obtained during basal anesthesia.
† $P < 0.05$. ‡ $P < 0.02$. § $P < 0.01$.

anesthesia we obtained the mean and standard error of the control response (arithmetic mean of the values obtained by CO_2 stimulation before and after administration of an anesthetic). Both 2.5 and 5.0 per cent CO_2 invariably caused proportional increases in sympathetic activity with the basal anesthetic. During inhalation of halothane sympathetic activity increased from 37.3 ± 10.5 to 52.3 ± 13.1 imps/sec with 2.5 per cent CO_2 ($P < 0.01$) and to 68.2 ± 15.8 imps/sec with 5 per cent CO_2. During administration of methoxyflurane sympathetic frequency increased from 62.8 ± 14.6 imps/sec to 90.8 ± 13.9 imps/sec with 2.5 per cent CO_2 ($P = 0.06$) and to 106.0 ± 13.8 imps/sec with 5 per cent CO_2 ($P < 0.05$). During cyclopropane anesthesia 2.5 per cent CO_2 caused an increase in frequency from 93.8 ± 11.0 imps/sec to 125.8 ± 11.3 imps/sec ($P < 0.001$) and 5 per cent CO_2 caused an additional increase to 150.0 ± 15.4 imps/sec ($P < 0.01$).

Table 2 also shows the effects of CO_2 on mean artreial blood pressure (MABP) during anesthesia with the three agents as well as with the basal anesthetic. The means and standard errors of control values (arithmetic mean of values obtained during CO_2 stimulation before and after an anesthetic) are shown under those obtained during exposure to an anesthetic. In every control trial 2.5 per cent CO_2 caused a small increase in arterial pressure, but increasing the CO_2 concentration to 5.0 per cent caused no further change in arterial pressure. Only with halothane anesthesia and only with 5 per cent CO_2 were the changes produced statistically significant ($P < 0.05$). The symbol "Δ" indicates ventricular arrhythmia. During halothane anesthesia arrhythmias were present in two cats before administration of CO_2 and in four after either 2.5 or 5.0 per cent CO_2. During cyclopropane anesthesia arrhythmias were present in four cats, and they developed in one other cat during the administration of CO_2. Methoxyflurane anesthesia, on the other hand, was not associated with cardiac arrhythmias even during administration of CO_2.

Table 3 shows the sympathetic nervous responsiveness to CO_2 as defined in the introduction, with control values calculated as arithmetic means of the initial and final CO_2-stimulation-trial values. It is evident that with none of the three anesthetics used was the sympathetic responsiveness to CO_2 different from that found during basal anesthesia.

Discussion

The results of early studies in man comparing sympathoadrenal responses to carbon dioxide in conscious subjects with those observed during anesthesia suggested that cyclopropane failed to reduce the effects of respiratory acidosis, but that halothane suppressed them.[1, 6] Since previous investigations relied on the measurement of circulating catecholamines the results were difficult to interpret, particularly because the resting levels in man are virtually indistinguishable from zero using extant methods. Therefore, a reduction in sympathetic nervous activity occurring in response to halothane would go undetected. Another source of difficulty is that postganglionic sympathetic nerves liberate norepinephrine into circulating blood only under conditions of moderate or intense activity, and the relation between liberation rate and impulse frequency may be alinear. In addition, the end-result of sympathetic activation depends not only on central responsiveness but upon the adequacy of ganglionic transmission, a variable not measured in the present study. Finally, the control observations in the human studies were far from pure pharmacologically, since CO_2 inhalation in conscious subjects produced intense discomfort which must have contributed to the sympathetic response. With these reservations in mind, the present results can be better interpreted.

Another difference is that the present experiments involve cats extensively dissected during light basal anesthesia. Although the wound was covered with warm mineral oil, there may have been some afferent sympathetic drive, and the administration of a more potent anesthetic could have reduced it simply by providing analgesia. It is possible that the failure of cyclopropane to elevate sympathetic nervous activity in this study stems from this cause—*i.e.*, direct stimulation by cyclopropane is counterbalanced by an indirect (analgesic) reduction in activity. However, there was no objective sign of arousal at any time in our animals. The pupils were small,

the nictitating membrane was relaxed, and piloerection was absent.

It is noteworthy that halothane markedly reduced central sympathetic activity and that, although the response to carbon dioxide was normal on a percentage basis, the maximal activity attained was no greater than the control (no halothane) level. If the change in impulse frequency caused by carbon dioxide had been considered instead of the percentage change (*cf.* table 2) the response would have been estimated as half-normal. Such a response, occurring in man, would have produced findings similar to those observed previously [1] using indirect methods.

The same considerations apply, although to a lesser degree, to the action of methoxyflurane. This agent inconsistently reduced the resting (zero inspired CO_2) level of sympathetic activity, and it reduced the absolute increase in frequency caused by carbon dioxide. On a percentage basis, however, the response during administration of methoxyflurane was little different from that seen in the absence of methoxyflurane.

Cyclopropane had a different effect. In four of the six cats this agent elevated sympathetic discharge, but in one cat there was no change and in one cat a decline occurred. The net effect of cyclopropane on sympathetic activity, therefore, was not statistically significant. Similarly, the response to carbon dioxide was entirely normal. This finding substantiates the conclusion drawn from our earlier studies in man.[1]

It is of interest that these anesthetics, and in particular cyclopropane, should fail to affect the sympathetic response to carbon dioxide in concentrations which severely depress both spontaneous respiration and the respiratory response to carbon dioxide. This difference may be attributable to a more rostral location of the respiratory apparatus within the central nervous system in comparison with that of the vasomotor center.

The measurement of arterial pressure as an indication of sympathetic nervous activity gains no support from our data. During basal anesthesia 2.5 per cent CO_2 usually caused an increase in arterial pressure, whereas 5.0 per cent CO_2 failed to increase it further despite the occurrence of a further increase in sympathetic activity at the higher level of CO_2 tension. Presumably the discrepancy results from direct peripheral actions of CO_2, namely vasodilatation or decreased myocardial contractile force or both.

When any of the three inhalational anesthetics studied here was given, the analysis based on blood-pressure responses became even more misleading. In fact, only during the administration of 5.0 per cent CO_2 to cats receiving halothane was there a significant increase in arterial pressure. One explanation for this was the occurrence of ventricular extrasystoles during inhalation of 5 per cent CO_2 in four of the six cats during halothane anesthesia and in five of the six during breathing of cyclopropane. Similar observations were made by us previously in man.[1] In contrast, none of the cats had premature ventricular contractions when given methoxyflurane, a finding in agreement with that of Black.[7]

An unexpected finding was that with halothane two cats and with cyclopropane four cats had ventricular extrasystoles before any CO_2 was given and that the occurrence of these arrhythmias correlated poorly with the sympathetic nervous response.

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