Alcohol and Regional Blood Flow in Brains of Rats (37725)

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Alcohol in large quantities is a potent cerebral vasodilator in man (1). This finding has been confirmed in the rat (2). On the other hand, small doses have been reported to be without effect on vascular resistance (3).

Although the amount of alcohol required to produce significant vasodilatation is large, it is nevertheless possible that smaller doses either produce very local changes which are diluted by a largely unreactive mass of tissue or produce a pattern of combined vasodilatation and vasoconstriction which would result in no obvious change in total cerebral blood flow. It is desirable to know whether the response to alcohol varies with region, and to what extent such changes may be related to pCO_2.

Until recently, inadequate or tedious methodology has limited the study of brain blood flow in general. By modifying a technique which has been used successfully to measure local blood flow in non-nervous tissues (4–6), the blood flow to various areas of the brains of conscious, unrestrained animals as small as rats now can be determined relatively simply and quickly (7).

We now report the effects on regional brain perfusion of ethanol at three dose levels: a low dose causing no behavioral changes, an intermediate dose producing ataxia but no loss of consciousness, and a high dose producing anesthesia.

Methods. The blood flow method is our modification of Sapirstein's indicator fractionation technique (4) permitting the use of unanesthetized animals; its principle is described elsewhere (7). Employing antipyrine-^{14}C as the indicator for nervous tissues, the flow fraction of the cardiac output perfusing a region is measured simultaneously with that of the cardiac output. The method, therefore, permits estimation, not only of the distribution of the cardiac output, but the minimum absolute flow of blood which exchanges nutrients with the region, as well. The basic equation of the indicator fractionation technique states that

$$U_i/I \simeq F_i/\text{C.O.}, \qquad (1)$$

whenever

$$\int_0^T Ca\,dt = \int_0^\infty C'a\,dt,$$

where U_i = indicator uptake in tissue mass i at time (T), I = body uptake of indicator, F_i = blood flow to tissue mass i, C. O. = cardiac output, and $C'a$ describes the indicator dilution curve obtained from the real one by substituting the extrapolated points after recirculation for the real ones.

Equation (1) states, in effect, that when an indicator such as antipyrine is administered in a single intravenous injection and the killing time is short, then the pattern of antipyrine distribution in the brain will be the same as the pattern of the fractional distribution of the cardiac output.

The main study was performed in adult male Wistar rats, which were 75–83 days of age. Three days before the measurement procedure was performed, PE-50 polyethylene catheters were implanted in one femoral vein, the opposite femoral artery, and in the peritoneal cavity. The blood vessel catheters were filled with heparin (1:1000) and heat sealed; all catheters were brought up under the skin of the flank, the back, and the dorsal

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[1] Deceased.

aspect of the neck and stored in a covered plastic cap which was stitched in place.

On the day of the measurement, the animals were placed in a small black lucite box at $T = -20$ min, allowed a calming period of 10 min, after which the catheters were freed from the neck cap. The exteriorized ends of the catheters, brought through slits in the box, were long enough to provide some slack when the animal moved about in the box. At $T = -10$ min, 20 ml saline or 20 ml saline solution containing 0.48, 2.4, or 4.8 g/kg of ethanol was administered through the peritoneal catheter. At the same time a 100-μl blood sample was collected from the arterial catheter for pH and blood gas determinations in a standardized Radiometer BMS-3 blood gas machine. At $T = -1$ min, another 100-μl sample of arterial blood was collected for blood-gas determination, and the venous catheter was loaded with 1.5 μCi of antipyrine-^{14}C (26 μg) in a volume of 40 μl. At $T = 0$, the label was flushed smoothly into the circulation with 120 μl of saline over a 1-sec period to minimize hemodynamic transients.

Also at $T = 0$, collection of 1-sec samples of arterial blood, about 15 μl each, was begun for the determination of the cardiac output by an indicator dilution technique (4) in which antipyrine replaced the previously employed ^{86}rubidium indicator. Collection of arterial blood ended at $T = 15$ sec. At $T = 20$ sec, the animal was killed by a rapid iv injection of 250 μl of a saturated KCl solution; the heart stopped within one beat.

In an additional group of 5 animals receiving the highest dose of ethanol, respiration was supported by means of a Harvard Rodent Respirator Pump and silicone rubber face mask.

The brain was removed immediately and dissected according to a protocol of Glowinski and Iversen (8). Brain parts were weighed in counting vials to which 15 ml of liquid scintillation solvent (Bray's) was added immediately after weighing. The vials were gently shaken for 2 hr and were then counted in a Packard Tri-Carb counter. Extraction of antipyrine into the scintillation solvent was essentially complete ($> 98\%$) after 1 hr agitation, as indicated by combustion analysis.

Results. At blood concentrations of ethanol ranging from 20 to 50 mg/100 ml, there were no changes in blood pH, pCO$_2$ or pO$_2$ (Table I). At this relatively low dose, *i.e.*, one-fifth the intoxicating dose for a rat or man, there were no regional changes in the blood flow to the brain.

At a dose which produced behavioral effects of intoxication, blood levels of ethanol ranged from 150 to 250 mg/100 ml. Blood pH decreased slightly while blood oxygenation improved. Some regions such as cerebellum and hippocampus showed decreased perfusion—the latter significantly ($p < 0.05$). On the other hand, blood flows to the basal ganglia, frontal cortex, and olfactory bulb increased slightly. However, even at this dose, flows to pons and medulla, and parietal and occipital cortex remained unchanged from control levels (Table II).

TABLE I. Differential Effects of Ethanol on Blood Characteristics in the Male Rat.

	Control	Ethanol (0.48 g/kg)	Ethanol (2.4 g/kg)	Ethanol (4.8 g/kg)
Cardiac output (ml/min/kg)	375 ± 22[a]	389 ± 18	388 ± 23	377 ± 18
Blood ethanol (mg/100 ml, range)		30–50	150–250	410–460
Arterial blood				
pH	7.41 ± 0.01	7.42 ± 0.01	7.37 ± 0.01†	7.30 ± 0.01‡
pCO$_2$ (mm Hg)	40 ± 2	36 ± 1	38 ± 2	50 ± 2 ‡
pO$_2$ (mm Hg)	86 ± 2	92 ± 3	98 ± 4 *	65 ± 4 ‡
Number of animals	12	12	9	10

[a] Values are means ± standard error. * $p < 0.05$, † $p < 0.005$, ‡ $p < 0.001$.

TABLE II. Differential Effects of Ethanol on Regional Brain Blood Flow in the Male Rat.

Tissue	Control	Ethanol (0.48 g/kg)	Ethanol (2.4 g/kg)	Ethanol (4.8 g/kg)
Cerebellum	0.92 ± 0.03[a]	0.86 ± 0.03	0.83 ± 0.03	0.97 ± 0.05
Pons and medulla	0.82 ± 0.03	0.79 ± 0.03	0.79 ± 0.04	0.90 ± 0.04*
Hypothalamus	0.85 ± 0.02	0.82 ± 0.04	0.82 ± 0.03	0.92 ± 0.05
Basal ganglia	0.86 ± 0.02	0.85 ± 0.04	0.92 ± 0.04	0.85 ± 0.05
Midbrain	0.89 ± 0.02	0.88 ± 0.03	0.86 ± 0.04	0.94 ± 0.04
Dorsal hippocampus	0.77 ± 0.02	0.72 ± 0.03	0.68 ± 0.02*	0.74 ± 0.04
Olfactory bulb	0.78 ± 0.03	0.75 ± 0.03	0.83 ± 0.03	0.95 ± 0.05†
Cortex frontal	1.00 ± 0.03	0.91 ± 0.03	1.11 ± 0.07	1.03 ± 0.06
parietal	1.06 ± 0.04	1.00 ± 0.04	1.08 ± 0.06	0.92 ± 0.05*
occipital	1.00 ± 0.03	0.94 ± 0.04	1.01 ± 0.04	0.91 ± 0.05

[a] Values are means ± standard error. * $p < 0.05$, † $p < 0.005$. Flow is expressed as ml/min × g tissue wet wt.

Twice the intoxicating dose, 4.8 g/kg, also doubled the blood levels of ethanol and clearly disturbed the blood gas picture; pH fell, pCO_2 rose, and pO_2 fell sharply. Unconsciousness occurred within 6 min after ip administration. Although blood flow values varied more widely than at lower doses, perfusion of the pons and medulla, and olfactory bulb rose by 10 and 22%, respectively, but decreased to the parietal and occipital cortical regions by 14 and 9%, respectively (Table II).

Hyperventilation occurred at the low dose and was especially obvious at the intermediate dose; ventilation rate was markedly depressed at the high dose. At this latter dose respiratory assistance by means of a pump and face mask was sufficient to return pO_2 and pCO_2 values to control levels; the acidosis, nevertheless, was not reversed (Table III). Under these circumstances, regional brain blood flow also was markedly altered.

TABLE III. Differential Effects of Anesthetic Doses of Ethanol and Pentobarbital on Regional Brain Blood Flow in the Male Rat.

Tissue	Control	Ethanol (4.8 g/kg) Unrespired	Ethanol (4.8 g/kg) Respired	Pentobarbital (40 mg/kg)
Cerebellum	0.92 ± 0.03[a]	0.97 ± 0.05	0.68 ± 0.02‖	0.89 ± 0.02
Pons and medulla	0.82 ± 0.03	0.90 ± 0.04*	0.66 ± 0.02§	0.85 ± 0.02
Hypothalamus	0.85 ± 0.02	0.92 ± 0.05	0.67 ± 0.03‖	0.80 ± 0.02
Basal ganglia	0.86 ± 0.02	0.85 ± 0.05	0.69 ± 0.04§	0.74 ± 0.02‖
Midbrain	0.89 ± 0.02	0.94 ± 0.04	0.72 ± 0.04‖	0.82 ± 0.02‡
Dorsal hippocampus	0.77 ± 0.02	0.74 ± 0.04	0.56 ± 0.03‖	0.67 ± 0.02§
Olfactory bulb	0.78 ± 0.03	0.95 ± 0.05§	0.71 ± 0.02	0.78 ± 0.02
Cortex frontal	1.00 ± 0.03	1.03 ± 0.06	0.82 ± 0.05‡	0.79 ± 0.02‖
parietal	1.06 ± 0.04	0.92 ± 0.05*	0.73 ± 0.05‖	0.88 ± 0.02‖
occipital	1.00 ± 0.03	0.91 ± 0.05	0.80 ± 0.07†	0.81 ± 0.02‖
Cardiac output (ml/min/kg)	379 ± 19	377 ± 18	297 ± 7 *	388 ± 10
Arterial blood				
pH	7.41 ± 0.01	7.30 ± 0.01‖	7.33 ± 0.01‖	7.38 ± 0.01§
pCO_2 (mm Hg)	40 ± 2	50 ± 2 ‖	38 ± 1	46 ± 1 ‖
pO_2 (mm Hg)	86 ± 2	65 ± 4 ‖	81 ± 2	66 ± 1 ‖
Number of animals	15	10	5	19

[a] Values are means ± standard error. * $p < 0.05$, † $p < 0.02$, ‡ $p < 0.01$, § $p < 0.005$, ‖ $p < 0.001$. Flow is expressed as ml/min/kg tissue wet wt.

Blood flows to most regions were depressed, especially to the hippocampus, cerebellum, and parietal cortex (Table III). The decreased perfusion of the brain was explained only partially by the lowered cardiac output.

It should be noted that the cardiac output did not change with any dose of ethanol and fell 21% only when respiration was assisted in the animal rendered unconscious by the highest dose.

Discussion. Regional blood flow responses to various doses of ethanol described here do not altogether support previously reported findings (1, 2). Short of a toxic dose, ethanol failed to significantly increase the perfusion of any brain region. On the other hand, at the dose causing general disorganization of motor behavior, 2.4 g/kg (blood content, 150–250 mg/100 ml), blood flow to the cerebellum and hippocampus was decreased. The characteristic loss of cerebellar functions at this dose may be related to a reported direct action of ethanol on cellular elements (11). Although similar effects on hippocampus have not been described, unusually high protein turnover in this region (13), coupled with the recent evidence that ethanol decreases protein turnover in cerebral gray matter (14), suggests that the hippocampus, too, may be selectively vulnerable to ethanol. It may be premature, but nevertheless useful, to speculate on this reduced flow to the cerebellum and hippocampus as evidence of the first functional changes presaging, what in man has been described as the Wernicke–Korsakoff disease. This disease, often associated with chronic alcoholic intoxication, is characterized by motor system defects and memory and learning deficits, particularly of recent events (15, 16). The animal model reported here, therefore, may find utility in exploring not only acute alcoholic intoxication, but the mechanism of chronic alcoholic disease, as well.

At the dose causing anesthesia, respiratory depression, and hypercarbia (4.8 g/kg), ethanol did elevate perfusion of most subcortical regions slightly, but significantly only to the pons and medulla, and olfactory bulb; flow to the parietal cortex was reduced (Table II). Battey *et al.* (1) observed a hyperemia in whole human brain during alcohol intoxication, which also was associated with respiratory depression and consequent hypercarbia. It is noteworthy that the similar occurrence of respiratory depression, low arterial pH, and high pCO_2 in animals anesthetized with pentobarbital (7) was not accompanied by cerebral hyperemia.

These results with the toxic ethanol dose were not expected in view of ethanol's ability to decrease the metabolism of brain tissue *in vitro* (10) and the oxygen utilization of brain in man (1). Assuming that blood flow is regulated by metabolic function (9), anesthesia should have been accompanied by reduced perfusion, as has occurred in the case of another depressant, pentobarbital (7), rather than by constant or improved perfusion.

Since the unusually high arterial pCO_2 and low pO_2 at this dose may have obscured the perfusion picture, an additional high dose group of animals received respiratory assistance sufficient to return the blood gas contents to control levels. Only then did the perfusion pattern conform to predictions (Table III); in general, ethanol, like pentobarbital (7), appeared to reduce regional brain perfusion. However, in comparing the fractional distributions of the cardiac output (flow fractions) in Table IV (which ignore the contributions of fluctuations in the cardiac output), differences between the effects of ethanol and pentobarbital are evident, as follows:

1. The flow fractions to all regions except the parietal cortex, and possibly hippocampus and cerebellum, remained constant in the ventilated, ethanol-anesthetized rat. The net reduction in regional blood flow, therefore, was due primarily to a decreased cardiac output. This suggests that one of ethanol's important effects in the brain may be inhibition of autoregulatory mechanisms.
2. In contrast, pentobarbital reduced the flow fraction delivered to most regions, particularly to the basal ganglia, midbrain, and frontal and occipital cortex.
3. Ethanol, if anything, improved perfusion of the olfactory bulb.
4. Whereas pentobarbital uniformly low-

TABLE IV. Fractional Distribution of the Cardiac Output to the Brains of Rats Anesthetized with Ethanol or Pentobarbital.

Tissue	Control	Ethanol (4.8 g/kg) respired[b]	Pentobarbital (40 mg/kg)[c]	
Cerebellum	0.77 ± 0.03[a]	0.68 ± 0.03	0.74 ± 0.03	
Pons and medulla	0.68 ± 0.03	0.67 ± 0.03	0.71 ± 0.03	
Hypothalamus	0.73 ± 0.03	0.68 ± 0.03	0.66 ± 0.02	*[d]
Basal ganglia	0.74 ± 0.04	0.69 ± 0.04	0.61 ± 0.02	‖
Midbrain	0.77 ± 0.04	0.73 ± 0.04	0.68 ± 0.02	†
Dorsal hippocampus	0.64 ± 0.03	0.56 ± 0.03	0.55 ± 0.02	§
Olfactory bulb	0.66 ± 0.03	0.71 ± 0.02	0.64 ± 0.02	
Cortex frontal	0.83 ± 0.04	0.83 ± 0.05	0.66 ± 0.02§	§
parietal	0.89 ± 0.04	0.73 ± 0.05*	0.73 ± 0.02	§
occipital	0.83 ± 0.04	0.80 ± 0.07	0.68 ± 0.02*	§
Number of animals	15	5	20	

[a] Values are means ± standard error. Flow fraction is expressed as percent/mg tissue wet wt.

[b] Ethanol condition compared to that of control.

[c] Pentobarbital compared to ethanol.

[d] Pentobarbital compared to control (see Table III, footnotes *, ‖, †, §).

ered the flow fraction delivered to the cortex by about 19%, ethanol reduced only the flow fraction to parietal cortex, leaving the perfusion of frontal and occipital cortex unaffected.

Since these differences suggest selective actions on regional brain metabolism and associated functions, ethanol may not be as general a nervous system depressant as indicated by McIlwain (10), nor as selective for neocortical functions as suggested by Hughlings Jackson. These observations bear further attention.

Finally, some mention should be made of other consequences of ethanol intoxication. Ethanol produces early alterations of ventilation in awake dogs (12). With increasing blood concentrations, there is a progressive decrease in tidal volume accompanied by an increase in rate. Ultimately the decreased tidal volume leads to CO_2 retention; arterial pO_2 also decreased. This appears to be the case for our rats, as well.

The fall in cardiac output seen in the respired ethanol-anesthetized rats also occurs in dogs (12) and suggests that much of the reported peripheral, as well as central, vasodilation associated with ethanol toxicity is dependent on an elevated arterial pCO_2. On the other hand, the acidosis found in both the hyperventilating, ataxic rat and the respired, unconscious rat must have been a metabolic acidosis, since it persisted in the absence of an elevated pCO_2.

Summary. Alcohol, in large amounts, increases the blood flow to brains of man and other animals. To what extent blood flow to specific regions of the brain varies with smaller, nonanesthetizing doses has been the subject of the present study. A new radioisotopic method was employed which permitted the simultaneous measurement of blood flow to each of ten regions of the brain in unrestrained, unanesthetized animals as small as rats. Three doses of alcohol were used: a low dose causing no behavioral change, an intermediate dose causing lack of coordination but no loss of consciousness, and a high toxic dose producing anesthesia. The blood levels of alcohol causing various signs of intoxication were the same in rats and man. At the low dose, no changes were seen in brain blood flow. At the intoxicating dose slight, but insignificant, increases were seen in the basal ganglia, frontal cortex, and olfactory bulb, while hippocampal blood flow decreased. At the anesthetic dose, blood flow increased to the olfactory bulb and the brain stem and actually decreased slightly to the parietal cortex. Support of the depressed ventilation rate observed at this dose of ethanol restored the pCO_2 and pO_2 values

to control levels but did not reverse the observed acidosis; cardiac output fell, signaling increased peripheral resistance. Under these circumstances, the flow of blood to all brain regions fell, most notably to the hippocampus, cerebellum, and parietal cortex. The effects of ethanol and pentobarbital are compared.

We are indebted to Dr. D. Knowlton for the analyses of blood levels of ethanol. This work was supported in part by a grant from The Licensed Beverage Industries and by Public Health Service Grant NB05526.

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Received July 13, 1973. P.S.E.B.M., 1973, Vol. 144.