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Ethanol Decreases Entry of Vincristine into Brain of Rat: Modification by Acetylcholine or Histamine (42712)

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Abstract. Male Sprague–Dawley rats were anesthetized with pentobarbital sodium and a jugular vein and femoral artery cannulated. Ethanol (3%; 13.3 ml/kg) was injected intraperitoneally 5 min before the administration of 10 μCi [^{3}H]vincristine sulfate intravenously. One minute later, saline, acetylcholine, (1 or 2 μg/kg) or histamine (1.25, 2.5 or 5 μg/kg) was given intravenously. At 15 min the thoracic cavity was opened, a cardiac sample of blood obtained, and saline infused into the left ventricle to remove blood from the brain. Samples of the cerebral cortex, midbrain, cerebellum, and plasma were subjected to liquid scintillation counting. The concentration of ethanol at 20 min after its administration was 20.3 mg/dl. This was associated with a significant decrease in radioactivity in the cerebral cortex and midbrain and a nonsignificant decrease in the cerebellum. Administration of 2 μg/kg of acetylcholine in the presence of ethanol decreased the blood pressure and increased the movement of radioactivity into the cerebral cortex and cerebellum while causing a significant decrease in the midbrain. Histamine (2.5 μg/kg) significantly increased the movement into the cerebellum and 5 μg/kg decreased the movement into the midbrain. The permeability of the blood–brain barrier to [^{3}H]vincristine was decreased by ethanol and this could be modified regionally by vasoactive doses of acetylcholine and histamine. Possible therapeutic advantage might result if vincristine were given in the presence of ethanol which should diminish the potential neurotoxicity.

Vincristine is an effective cancer chemotherapeutic agent that often is associated with neurotoxicity (1). If its entry into the central nervous system could be decreased, the number of people who could be treated as well as the systemic dose could be increased. Ethanol has been studied extensively for its effect on the function of the blood–brain barrier (BBB) and has been found to increase (2–7), not change (8, 9), and decrease (10–12) the penetration of a variety of substances. Thus, it was likely to alter the permeability of the BBB to vincristine. We have previously found that acetylcholine and histamine increased the permeability of the BBB to albumin and sodium pertechnetate (13). We therefore thought it would be of interest to determine whether acetylcholine or histamine would alter the permeability of the BBB in the presence of ethanol.

Materials and Methods. Male Sprague–Dawley rats from the Charles River colony weighing 258 to 386 g were anesthetized with 50 mg/kg pentobarbital sodium intraperitoneally. The anesthesia was supplemented when necessary. A jugular vein was cannulated with PE-10 polyethylene tubing to permit the administration of saline, drugs, and radioactivity. A femoral artery was cannulated to permit the recording of the mean arterial blood pressure via a P-1000-A pressure transducer connected to a Narco Bio-Systems physiograph (DMP-4A). The permeability of the BBB was evaluated using [G-^{3}H]vincristine sulfate 2–10 Ci/mmole (Amersham). Approximately 10 μCi of vincristine sulfate in saline was given intravenously 5 min after the intraperitoneal administration of saline or ethanol (3%) in saline (13.3 ml/kg). One minute after the administration of the radioactivity, saline, acetylcholine (1 or 2 μg/kg), or histamine (1.25, 2.5 or 5 μg/kg) was given intravenously. Fifteen minutes after the administration of the vincristine the thoracic cavity was opened and a sample of blood was obtained from the right cardiac ventricle and it was centrifuged immediately. The venae cavae were severed and 125 ml of 0.9% saline was infused into the left ventricle to remove blood from the cerebral vasculature. The brain was removed and divided into three

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portions, viz. cortex, midbrain, and cerebellum. Approximately 50 mg of each region of the brain and plasma was placed in scintillation vials to which 2 ml Soluene was added to dissolve the tissue. Then 8 ml Multisol was added and mixed thoroughly prior to counting in a Beckman LS-230 liquid scintillation counter. The samples were counted three times each and background was subtracted from the mean of the three counts. The ratio of the counts $\cdot$ min^{-1} g^{-1} in brain to that in plasma was used as a measure of the permeability of the BBB in groups of four to seven rats each. The concentration of ethanol in 25 μl of plasma was determined with a Sigma diagnostic kit and quantified using a Gilford 240 spectrometer at 340 nm. The results of each treatment were compared with saline-treated controls using the Student t test (14).

Results. The mean arterial blood pressure of 38 rats was 101 ± 0.6 mm Hg. The mean concentration of ethanol in the plasma of these animals 20 min after its administration intraperitoneally was 20.3 ± 1.0 mg/dl.

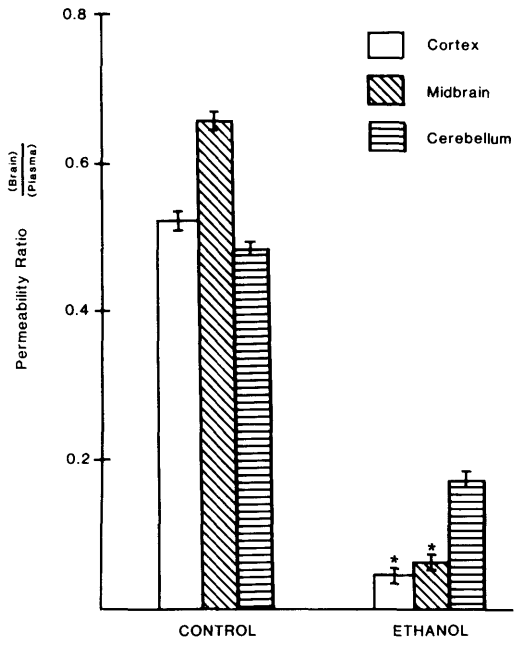

FIG. 1. The permeability ratio of [^{3}H]vincristine in brain to that in plasma in the cerebral cortex, midbrain, and cerebellum of rats given saline (control) or 3% ethanol (13.3 ml/kg) intraperitoneally 5 min before [^{3}H]-vincristine intravenously and 20 min before sacrifice. *Indicates significantly different ($P < 0.05$) from control.

TABLE I. EFFECTS OF ACETYLCHOLINE AND HISTAMINE ON THE MEAN ARTERIAL PRESSURE OF RATS PRETREATED WITH ETHANOL

Drug	Dose (μg/kg)	Decrease in mean arterial pressure (mm Hg ± SEM)
Acetylcholine	1 (7)	2 ± 1.6
	2 (5)	26 ± 7.4
Histamine	1.25 (5)	0
	2.5 (5)	1.6 ± 0.9
	5.0 (5)	11 ± 2.4

Note. Numbers in parentheses indicate the numbers of animals in the group. The values were the maximal change in pressure in the minute following intravenous administration.

The effects of ethanol upon the penetration of [^{3}H]vincristine into the brain are illustrated in Fig. 1. The ethanol caused a marked decrease in the concentration of the alkaloid in the three regions of the brain that were evaluated. This decrease was statistically significant for the cortex and midbrain and nearly so for the cerebellum.

The administration of saline or ethanol intraperitoneally did not cause a change in the mean arterial blood pressure. The effects of acetylcholine and histamine on mean arterial blood pressure of rats given ethanol are summarized in Table I. Both drugs caused a dose-dependent decrease in the blood pressure. When the effects of these vasoactive substances on the penetration of [^{3}H]-vincristine through the BBB were determined, the results listed in Table II were obtained. There were drug-induced changes that were regionally specific. The higher dose of acetylcholine caused a significant increase in the movement of vincristine into the cortex and cerebellum and a significant decrease in the midbrain. The intermediate dose of histamine caused a significant increased uptake into only the cerebellum whereas the highest dose decreased the uptake only in the midbrain. Thus, regional selectivity of uptake occurred with both drugs.

Discussion. The ethanol caused a dramatic decrease in the uptake of [^{3}H]vincristine by the brain of the rat in a concentration in the plasma that would cause only threshold changes in behavior (1). This would agree with the reports of a number of authors who have found that ethanol decreased the up-

TABLE II. THE EFFECTS OF ACETYLCHOLINE AND HISTAMINE ON THE MOVEMENT OF [^{3}H]VINCRISTINE THROUGH THE BLOOD–BRAIN BARRIER OF RATS IN THE PRESENCE OF ETHANOL

Drug	Dose μg/kg	Permeability of blood–brain barrier		
		Cortex	Midbrain	Cerebellum
None	—	0.044 ± 0.016	0.0633 ± 0.013	0.088 ± 0.024
Acetylcholine	1	0.0728 ± 0.019	0.0303 ± 0.0133	0.118 ± 0.047
	2	0.108 ± 0.029*	0.0219 ± 0.0104*	0.149 ± 0.054*
Histamine	1.25	0.0328 ± 0.0188	0.0975 ± 0.0464	0.121 ± 0.037
	2.5	0.0624 ± 0.0342	0.0973 ± 0.0318	0.180 ± 0.036*
	5.0	0.0459 ± 0.0083	0.0249 ± 0.0133*	0.0866 ± 0.0265

Note. All animals had received ethanol (3%) (13.3 ml/kg) intraperitoneally 6 min before the administration of the drug. Permeability of the BBB is the ratio of counts · min^{-1} g^{-1} of brain/counts · min^{-1} g^{-1} plasma.
* Value is significantly different from control ($P < 0.05$).

take of α-aminobutyric acid (10), thiamine (11), and mannitol (12). Such a diverse group of chemicals would suggest that the effect is relatively nonspecific. This could be due to its effect on membranes where it has been found to increase fluidity and reduce movement of substances across the membrane (15–17). Alternatively, a change in regional blood flow, and hence delivery of the drug to the site of transfer, could also contribute to the results found in the present experiments. Such a selective effect on regional blood flow with no change in total cerebral blood flow has been reported with ethanol in rats (18). Both of these factors could be involved in the decreased permeation of vincristine found in the present experiments.

Earlier reports (19, 20) using [^{3}H]-vincristine have shown that the concentration in the blood decreases quite rapidly initially. The initial half-life was approximately 6–15 min. The subsequent half-life of elimination varied from 75 to 190 min. There was also a rapid rate of metabolism so that even in the 15 min of the present experiment there were appreciable proportions of radiolabeled metabolites to be expected. Further experiments would be necessary to determine whether the effects of ethanol reported here are on the movement across the BBB of the parent compound and/or the metabolites. Whether the effects of ethanol on the movement are cumulative would require the use of more intervals for sampling following the administration of the [^{3}H]vincristine.

Advantage might be taken of this effect of ethanol on the uptake of vincristine by the brain. Administration of the drug following ingestion of relatively small amounts of ethanol might decrease the movement of vincristine across the BBB sufficiently that the incidence of neurological toxicity would be decreased (1).

In earlier experiments from this laboratory (13) we had found that both acetylcholine and histamine increased the permeability of the BBB to albumin and sodium pertechnetate. This increase occurred even though the systemic blood pressure was decreased dose dependently, as in the present experiments. Numerous reports had linked an increased permeability to proteins or large molecular weight dextrans to acute changes in blood pressure (21–27). However, we have found a number of other dissociations between changes in systemic blood pressure and changes in permeability of the BBB (13, 28–31). Thus, the increases found in the cerebral cortex and cerebellum in the present experiments provide another example of this dissociation. That it is not an universal effect is exemplified by the decreases found in the midbrain with strongly vasodepressor doses of both acetylcholine and histamine.

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