Medical Therapies for Mood Disorders Alter the Blood-Brain Barrier

Abstract. *The effects of amitriptyline, lithium, and electroconvulsive shock on cerebral permeability and blood flow were tested. These three treatments share in common (i) the ability to influence the functional activity of central adrenergic neurons by way of effects on the release, reuptake, or metabolism of norepinephrine and (ii) therapeutic efficacy in mood disturbances. Under control conditions, cerebral permeability increases linearly with increasing arterial partial pressure of carbon dioxide and hence cerebral blood flow. All three treatments altered this relationship in a manner consistent with their adrenergic effects. Amitriptyline potentiated this increase in cerebral permeability whereas lithium and electroconvulsive shock blunted this phenomenon. These results support the hypothesis that one function of central adrenergic neurons is regulation of the blood-brain barrier and raise the possibility that a related effect may underlie the clinical usefulness of such treatments.*

Central adrenergic neurons have been the focus of extensive research in the neurosciences and psychiatry. Despite being implicated in a wide range of complex behaviorial phenomena (for example, appetite, sleep, memory, mood disorders), controversy surrounds what central functions they normally subsume. According to the central adrenergic vasoregulatory hypothesis, one function of these neurons is the regulation of blood-brain barrier permeability and cerebral blood flow (CBF). A link between this hypothesis and the catecholamine theory of mood disorders has been suggested by the finding in laboratory animals that tricyclic antidepressants alter the blood-brain barrier, increasing the diffusion of compounds such as water and ethanol into the brain (*1*). The catecholaminergic actions of these drugs apparently mediate this cerebrovascular effect (*2*), just as they are presumed to underlie the antidepressant effects of these drugs.

To further test this vasoregulatory hypothesis, we examined the cerebrovascular effects of electroconvulsive shock (ECS) and lithium in rats. Unlike tricyclic antidepressants, lithium and ECS attenuate the actions of central adrenergic neurons. On the basis of their central adrenergic effects, we correctly predicted that these two treatments would also alter the blood-brain barrier but in a manner different from that of the tricyclic antidepressants. The findings reported here support the central adrenergic vasoregulatory hypothesis. Moreover, they suggest that a common mechanism may exist to explain the parallel between the differential therapeutic efficacy of these treatments in affective disorders and their effects on the cerebromicrovasculature.

A double-labeling technique was used to measure the extraction of water, E_w. Water is diffusion-limited across the blood-brain barrier, whereas butanol is freely permeable (*3*). The E_w was determined by comparing the incomplete extraction of ^3H_2O to the complete extraction of [^{14}C]butanol, according to the equation

$$E_w = \frac{(^3H_2O/[^{14}C]\text{butanol})_{brain}}{(^3H_2O/[^{14}C]\text{butanol})_{blood}} \quad (1)$$

For all of the experiments we used Sprague-Dawley male rats (250 to 300 g). After initial anesthesia was induced by chloroform, a tracheotomy was performed. The animals were then paralyzed by *d*-tubocurarine. Anesthesia and respiration were maintained by means of a Harvard small animal respirator to deliver a mixture of 66 percent nitrous oxide and 34 percent oxygen, controlled by a small-volume gas mixer (Aalborg Instruments). Since the animals were mechanically ventilated, blood gases and *p*H values were a function of the ventilator settings and the gas mixture. Stroke volume was kept at 3.5 ml, but respiratory rate varied from 35 to 60 per minute. Blood gases were monitored in all the animals. The mean value (± standard error) for the arterial pressure of oxygen, P_aO_2, was 130 ± 6 mmHg. The *p*H and P_aCO_2 ranged from 7.18 to 7.51 and 16 to 55 mmHg, respectively. Body temperature was maintained at 37° ± 0.5°C by means of a rectal thermistor and electric lamp. The right femoral artery and vein were catheterized in all the rats to permit arterial sampling, tracer administration, and arterial blood pressure monitoring.

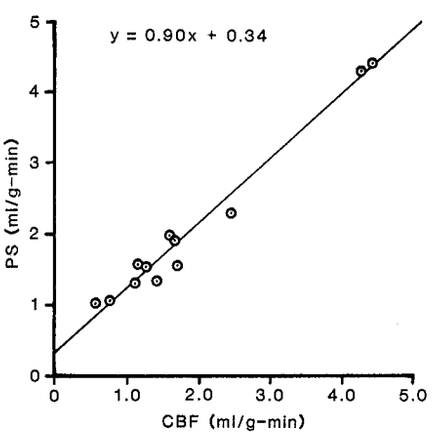

Fig. 1. Relation between the effective permeability of water across the blood-brain barrier (*PS*) and cerebral blood flow (*CBF*). The CBF was manipulated by varying respirator rates, producing arterial PCO_2 ranging from 18 to 55 mmHg. Arterial PO_2 was maintained constant for all subjects. Each point represents simultaneously measured *PS* and CBF from an individual animal. Under control conditions, *PS* increases linearly with CBF (r = .98, $P < .001$). No regional differences were observed. The data shown are based on total forebrain values (that is, diencephalon and telencephalon).

For these experiments, a bolus containing ^3H_2O and [^{14}C]butanol was injected in the femoral vein. After one complete cerebral transit time (10 seconds), the animals were killed by decapitation and the brain was removed. The brains were divided into the following regions by freehand dissection: rostral telencephalon, caudal telencephalon, diencephalon, cerebellum, and medullapons. These regions were dissolved in a tissue solubilizer (Protosol) and counted by standard dual-label scintillation techniques. An arterial blood sample provided the reference ratio that was used as the denominator in Eq. 1. The details of the method have been reported (*1*).

Cerebral blood flow was measured by withdrawing, at a uniform and predetermined rate, an arterial blood sample from a femoral catheter (*4*). This sample was obtained from 1 second prior to the injection of the tracer bolus until decapitation. This arterial sample was prepared and counted in the same manner as the brain samples. The CBF was then determined according to the equation

$$CBF = \frac{([^{14}C]\text{butanol})_{brain}}{([^{14}C]\text{butanol})_{blood}} \times \text{sampling rate} \quad (2)$$

This method has been validated by comparing its results to those obtained when CBF is measured simultaneously in the same animal by microsphere techniques and by physiologically altering CBF by CO_2 administration (*4*).

The amount of a diffusion-limited tracer, such as water, which will cross the blood-brain barrier after a single circulation through the cerebral capillary bed is determined by three variables: (i) the permeability coefficient of the substance

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(*P*), (ii) the surface area of the capillary (*S*), and (iii) CBF. The effective permeability (*PS*) of water is mathematically related to E_w and CBF. After E_w and CBF were measured, *PS* was calculated according to the equation derived from Renkin and Crone (*5*)

$$PS = -\ln(1 - E_w) \cdot \text{CBF}$$

Amitriptyline (17.5 mg/kg) was administered intraperitoneally to 12 rats. E_w and CBF were measured 15 minutes after drug administration. This dose and time were based upon our previous studies (*1*). Lithium chloride (1.6 mEq/kg) was administered intraperitoneally to 12 rats. E_w and CBF were measured 60 minutes after drug administration. After the animals were decapitated, trunk blood was obtained and assayed for lithium by means of flame ionization. Mean serum concentration (± standard error) was 1.49 ± .26 mEq/liter. The therapeutic range in man is 0.8 to 1.5 mEq/liter.

Twelve rats received a single ECS treatment consisting of a 1-second stimulus of 65 mA delivered by way of ear clips (*6*). The ECS treatments were first tested on nonparalyzed animals to ensure that they induced seizures. To test the effect of ECS on the blood-brain barrier, the treatments were given after the animal had been paralyzed and placed on the respirator. E_w and CBF were measured 15 minutes after ECS administration. There was no change in blood pressure or gases as a result of the ECS. An additional six animals received a course of one ECS treatment per day for 8 days. E_w and CBF were measured 15 minutes after their last treatment. A standard course of ECS for treatment of affective illness consists of six to ten treatments.

There were no group differences in E_w, CBF, or *PS* among the three control groups run concomitantly with each of the three studies. The results were therefore pooled to give a cumulative control group of 12 subjects. Under control conditions, *PS* was linearly related to CBF (Fig. 1). This finding replicates previous work in this laboratory demonstrating that *PS* increases with increasing P_aCO_2 and hence with increasing CBF (*4*). This observation suggests that the brain has two mechanisms for ensuring fluid balance—regulation of brain permeability and of CBF.

All three treatments altered this *PS* versus CBF relationship (Fig. 2). Amitriptyline augmented the increase in *PS* observed normally at higher CBF. According to one theory concerning their mode of action, tricyclic antidepressants increase the concentration and hence the postsynaptic effect of norepinephrine by blocking its reuptake into neurons. Previous work suggests that one function of the central adrenergic system is regulation of blood-brain barrier permeability and CBF (*1, 2*). The current results are compatible with both theories. Amitriptyline appears to potentiate the tonic influences of a neural system that functions, at least in part, to adjust barrier permeability for metabolic-induced changes in CBF. In related work, we have blocked the amitriptyline effect by ablating central adrenergic neurons or by giving central adrenergic antagonists (*7*).

Both lithium and ECS produced changes in the opposite direction, that is, blunted *PS* response to increased CBF. Lithium retards the liberation of norepinephrine from adrenergic neurons and enhances its reuptake (*8*). Both actions would reduce postsynaptic norepinephrine concentration. Knowledge of the effects of ECS on central adrenergic neurons is more limited. However, ECS is reported to depress central levels of norepinephrine, enhancing its conversion to normetanephrine within 10 to 15 minutes of a single treatment (*5*). Both lithium and ECS treatment should, therefore, blunt the actions of central adrenergic neurons. The results are consistent with the amitriptyline findings and the adrenergic vasoregulatory hypothesis.

There is an intriguing parallel between the differential therapeutic effects of these treatments and their effects on the blood-brain barrier. All these treatments are useful in treating depression. In mania, both ECS and lithium are efficacious, whereas tricyclic antidepressants aggravate the condition. Other workers have reported a reduction of CBF in depressed patients and increased CBF in manic patients (*9*). In the studies reported here, all three treatments produced an increase in *PS* at low CBF (Fig. 2). At high CBF, ECS and lithium blunted the increase in *PS* normally seen, whereas amitriptyline continued to potentiate this response (Fig. 2). All three treatments were given in a manner directly analogous to the clinical situation. With regard to amitriptyline, our previous work has

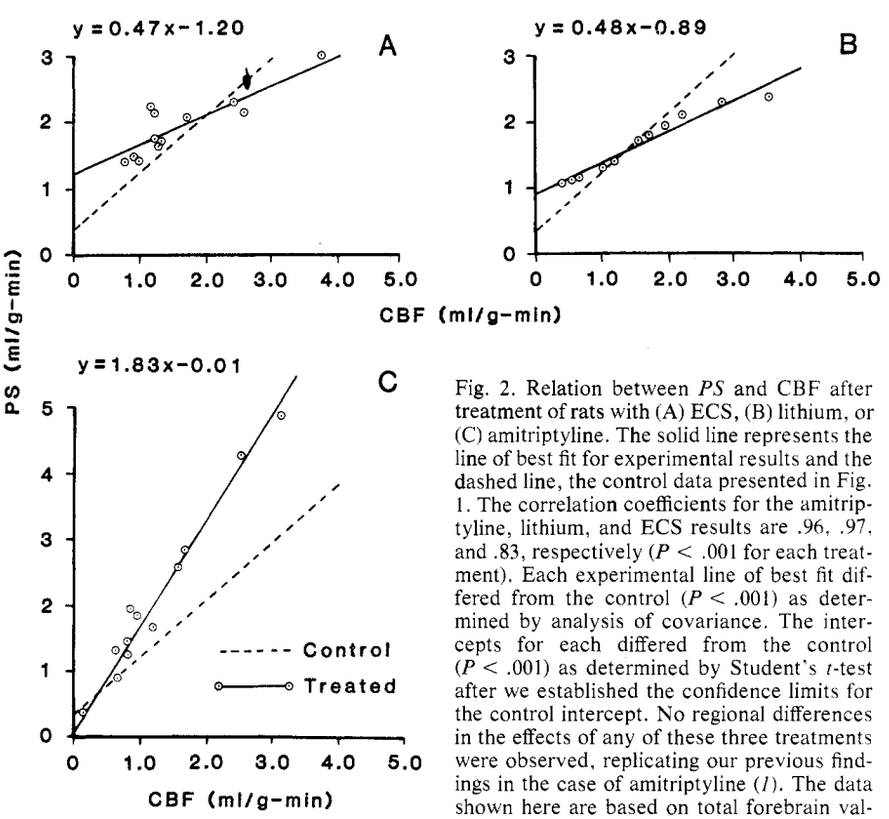

Fig. 2. Relation between *PS* and CBF after treatment of rats with (A) ECS, (B) lithium, or (C) amitriptyline. The solid line represents the line of best fit for experimental results and the dashed line, the control data presented in Fig. 1. The correlation coefficients for the amitriptyline, lithium, and ECS results are .96, .97, and .83, respectively ($P < .001$ for each treatment). Each experimental line of best fit differed from the control ($P < .001$) as determined by analysis of covariance. The intercepts for each differed from the control ($P < .001$) as determined by Student's *t*-test after we established the confidence limits for the control intercept. No regional differences in the effects of any of these three treatments were observed, replicating our previous findings in the case of amitriptyline (*1*). The data shown here are based on total forebrain values (that is, pooled results from diencephalon and rostral and caudal telencephalon). Long-term treatment with ECS—one treatment daily for eight consecutive days—did not alter the effects of acute ECS treatment. Essentially the same linear regression equation was obtained from six animals whose *PS* and CBF were measured 15 minutes after their eighth ECS treatment as from animals who received only a single ECS treatment: $y = 0.51x + 1.28$ and $y = 0.47x + 1.20$, respectively. The correlation coefficient for the results of long-term treatment was .85 ($P < .001$). Again, the effect was generalized and no regional differences were observed. These ECS results are similar to our previous findings with amitriptyline showing that the effect on the blood-brain barrier persists when long-term treatment patterned after the clinical situation is given (*1*).

shown that this effect (i) occurs at concentrations similar to those found to be therapeutic in patients, (ii) occurs in primates as well as rodents, and (iii) is transient, lasting less than an hour after a single administration but can be sustained more than 12 hours with long-term treatment similar to that needed for antidepressant response.

These results have both basic and clinical implications. First, three quite different treatments, all capable of altering central adrenergic neurons, alter cerebral fluid dynamics. Second, they have heuristic value, bringing together two discrete hypotheses: the catecholamine theory of affective illness and the central adrenergic vasoregulatory hypothesis proposed by Hartman and colleagues (*2*). This report indicates that the blood-brain barrier is under the influence of the central adrenergic system and suggests that such homeostatic regulation may be involved in the pathophysiology of affective illness. What exactly is altered (for example, brain water, ions, substrates) of importance to these illnesses remains to be defined. However, the failure of such a neurohumoral system capable of tonically influencing other cerebral tissue and cerebral homeostasis could explain how the global functional impairment—that is, mood alterations, cognitive and psychomotor changes, and disturbances of sleep, appetite, and energy—seen in affective disorders could occur and yet fully remit without residual cerebral tissue pathology.

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Possible Adaptive Value of Water Exchanges in Flexible-Shelled Eggs of Turtles

Abstract. *Use of energy reserves by embryos of common snapping turtles (*Chelydra serpentina*) is related to the hydric conditions to which eggs are exposed during incubation and to the net exchanges of water through the eggshells. Embryos developing inside eggs with a relatively favorable water balance use more of their energy reserves metabolically and grow larger before hatching than embryos inside eggs with less favorable water exchanges.*

Both laboratory studies (*1–4*) and field studies (*5*) indicate that the flexible-shelled eggs of many species of turtles exchange water with surrounding air and soil, but there is no consensus concerning the significance of this phenomenon (*6*). We report that the use of energy reserves by embryos of common snapping turtles (*Chelydra serpentina*) is related to the net exchange of water in incubating eggs and that hatchlings from eggs incubated in favorable hydric conditions are larger than turtles hatching from eggs incubated in less favorable settings. Because survival of young of other species of turtles is positively correlated with body size (*7*), the exchanges of water experienced by flexible-shelled eggs of turtles during natural incubation may have adaptive significance.

Eggs collected from two fresh nests (*8*) were incubated in covered containers at 29°C. Inside the containers, randomly selected eggs from both clutches were either half-buried in substrates, where water potentials were −130, −375, or −610 kilopascals (kPa) (*2*), or incubated on wire platforms above the substrates (*9*). Egg mass was measured on day 1 of incubation and then weekly through day 50; hatching began on day 56 (*8*). Small amounts of water were added to the substrates twice weekly to maintain relatively constant water potentials (*2*).

Changes in egg mass were similar to those reported for eggs of painted turtles (*Chrysemys picta*) (*2*). Eggs in the substrates generally increased in mass during the first half of incubation (indicating that eggs experienced net water absorption) and decreased in mass during the second half (indicating that eggs experienced net water loss). Eggs on the platforms generally experienced only small changes in mass during the first half of incubation and large declines in mass during later stages of development. The overall change in egg mass between days 1 and 50, which can be used as an index to hydric balance, was related both to the water potentials in the substrates and to placement of eggs on platforms or in substrates (Table 1).

After hatching, measurements were taken of the live mass, carapace length, carapace width, and head width of young turtles (Table 1). The hatchlings were frozen and their carcasses, including residual yolk withdrawn into the abdominal cavity before hatching, were dried to constant mass at 60°C (Table 1). Because none of these measures of mass or linear dimension is necessarily a reliable index to size (*10*), we submitted the data to a principal components analysis and generated a size index for each hatchling that summarized the variation in all of the measured variables (*10, 11*). The original data and the computed size indices were then examined by separate two-way analyses of covariance (*12*); substrate water potential and placement of eggs in substrates or on platforms were the fixed factors, and egg mass on day 1 was a covariate (*13*). The covariate was used to reduce variation in the data stemming from differences in the size of the eggs in which the turtles developed (*2, 3, 9*).

Live mass, linear dimensions, and size indices for hatchlings that emerged from eggs incubated in contact with the substrate were invariably larger than comparable values recorded for turtles hatched from eggs incubated on wire platforms in the same containers ($P \leq .001$) (Table 1

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