One can rationalize this result by considering that the normal fate of free adriamycin is to be taken into the cell and thus removed from interaction with the cell surface. In contrast, immobilized drug cannot enter the cell and remains available for interaction at the surface, which appears to represent the most sensitive drug target.

These experiments do not rule out the possibility that administration of native adriamycin affects cell viability through DNA intercalation. Also, it is possible that free or immobilized adriamycin could lead to the formation of toxic activated oxygen species, as suggested by Bachur *et al.* (*15*). We have shown, however, that a drug heretofore thought to have intracellular DNA as its major site of action can exert its biological activity solely by interaction with the cell surface. If adriamycin does not have to enter cells to be effective, then some form of nonpenetrating adriamycin might be used to avoid the metabolic repercussions of free drug administration. Consequently, it may be important to consider the cell surface as a promising target in the design of a new generation of anticancer agents.

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Antidepressants Alter Cerebrovascular Permeability and Metabolic Rate in Primates

Abstract. *External detection of the annihilation radiation produced by water labeled with oxygen-15 was used to measure cerebrovascular permeability and cerebral blood flow in six rhesus monkeys. Use of oxygen-15 also permitted assessment of cerebral metabolic rate in two of the monkeys. Amitriptyline produced a dose-dependent, reversible increase in permeability at plasma drug concentrations which are therapeutic for depressed patients. At the same concentrations the drug also produced a 20 to 30 percent reduction in cerebral metabolic rate. At higher doses normal autoregulation of cerebral blood flow was suspended, but responsivity to arterial carbon dioxide was normal.*

In studies on rodents, tricyclic antidepressants have enhanced the ability of simple substances such as water and ethanol to cross the blood-brain barrier (*1*). Several observations suggested that this drug action might be clinically relevant (*1*): (i) it occurs at concentrations which are therapeutic for depressed patients, and (ii) chronic administration—patterned after the clinical use of the drugs—produces a sustained and heightened effect. We report that amitriptyline hydrochloride (AMI) produces this alteration in primates as well as in rodents and that the increase in the brain extraction of diffusion-limited substances is primarily the result of a drug-induced increase in cerebrovascular permeability.

The brain extraction of water (*E*)—that is, the percentage of a bolus of radiolabeled water which passes from the vascular compartment into the brain in a single transit through the cerebral microcirculation—is dependent on the permeability coefficient of water across the cerebral capillary (*P*), the mean capillary surface area (*S*), and cerebral blood flow (CBF). The relation of *E* to these variables is expressed by the equation

$$\ln(1 - E) = -PS/\mathrm{CBF} \qquad (1)$$

developed by Renkin and Crone (*2*). The *PS* product is the effective permeability of the blood-brain barrier to the tracer. At physiological flows in the monkey, it appears to depend primarily on changes in *P* (*3*).

Six adult rhesus monkeys (*Macaca mulatta*) weighing 6 to 8 kg were anesthetized with phencyclidine (2 mg/kg, intraperitoneally), paralyzed with gallamine, and passively ventilated with 100 percent oxygen. End-tidal CO_2 pressure, arterial blood pressure, and rectal temperature were continuously monitored.

A femoral artery was cannulated and a catheter advanced to the level of the right internal carotid artery; this position was confirmed fluoroscopically. Through this catheter, 0.2 ml of whole blood labeled with [^{15}O]water was injected into the cerebral microcirculation. The extraction of the tracer bolus into the brain (*E*) and the rate of its washout (CBF) were measured by external detection of the annihilation radiation produced by ^{15}O. Because of its short half-life, sequential injections of ^{15}O-labeled water permitted assessment of changes in *E* and CBF due to the treatment, and these were used in Eq. 1 to calculate changes in *PS* (*3*).

In the first monkey, AMI (10 mg/ml) was administered (7 mg/kg) at 1 minute (Fig. 1). Two additional injections of AMI (3.5 mg/kg) were made at 15 and 25 minutes. The initial injection produced a prompt 22 percent increase in *PS* without altering CBF or mean arterial blood pressure (MABP). Permeability returned to normal within 10 minutes. The second injection of AMI caused a sustained 60 percent increase in *PS* with MABP remaining above 90 mmHg. After the third injection, *PS* dropped but remained above pretreatment values, MABP dropped to 80 mmHg and remained stable, and CBF decreased to 55 percent of baseline.

This decrease in CBF suggests drug-induced suspension of normal CBF autoregulation because in the rhesus monkey CBF is normally independent of MABP until MABP drops below 60 mmHg (*4*). The reactivity of the cerebral vasculature was tested in two ways: (i) 5 percent CO_2 was added to the inspired gas mixture 83 minutes after the first AMI injection, and this produced an increase in CBF without altering MABP; (ii) angiotensin II was administered intravenously at 108 minutes, and this agent produced a transient but marked increase in MABP and CBF (Fig. 1). These results suggest that even though CBF remained responsive to cerebral metabolic demands, as shown by increasing arterial pressure of CO_2, normal autoregulation (CBF is independent of perfusion pressure over a wide range of pressures) was suspended in this subject. Despite the injection of angiotensin II and administration of 5

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percent CO_2, *PS* remained elevated above pretreatment values for more than 100 minutes after the second dose of AMI.

In the other five monkeys the effect of a single interperitoneal injection of AMI (7.5 mg/kg) was studied. The AMI produced a 40 ± 5 percent mean increase in *PS* in all five monkeys (Fig. 2). In four, the maximum increase was observed within 20 minutes. A reduction in MABP occurred in these animals, but the reduction in CBF was modest (Fig. 2). In two animals, cerebral oxygen utilization (*5*) decreased by 20 to 40 percent within 10 minutes of AMI injection (Fig. 2), and the reduction persisted for more than an hour. Again, AMI did not alter blood gases.

Analysis of plasma samples obtained from the five animals 60 minutes after AMI administration (*6*), showed that the total tricyclic antidepressant–AMI plus nortriptyline–plasma concentration was 188 ± 33 ng/ml (mean ± standard error of the mean). One animal with only AMI in his plasma demonstrated the increase in *PS*, indicating that AMI rather than a metabolite is responsible for this effect.

These results provide additional evidence that AMI can enhance the brain uptake of diffusion-limited substances such as water in primates as well as rodents. This effect was dose-dependent, generalized, and reversible. The results then suggest that AMI influences homeostatic mechanisms which control the effective permeability of the blood-brain barrier.

In earlier studies of primates, electrical or chemical stimulation of the locus coeruleus, the major nucleus of the central adrenergic system, caused an increase in *PS* similar to that observed after administration of AMI, whereas central adrenergic antagonism by intraventricular administration of phentolamine produced a decrease (*7*). Although the mechanism of the AMI-induced increase in permeability was not tested, the dose-response data suggest that it is mediated by the central adrenergic system. A curvilinear relation is obtained when the percent change in permeability is plotted as a function of cumulative dose, using the three data points from the first animal (Fig. 1) and the average value from the other five (Fig. 2). A similar biphasic relation between brain concentration of AMI and the effect of the drug on the blood-brain barrier was described in rats (*1*). In both cases, the relation is compatible with an effect on the central adrenergic system. At low concentrations, AMI acts as an indirect agonist by facilitating the release of nor-

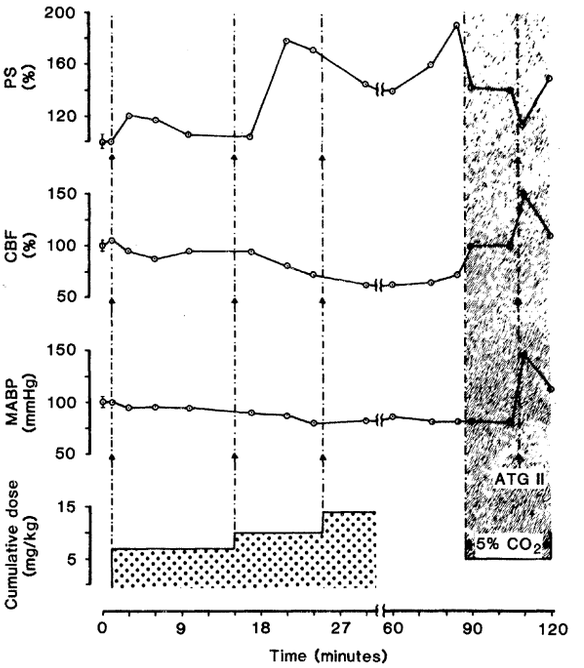

Fig. 1. Changes in cerebrovascular permeability (*PS*), cerebral blood flow (CBF), and mean arterial blood pressure (MABP) after three doses of AMI (cumulative dose). The value at 0 minute represents the mean (±S.D.) for four baseline measurements. The drug produced an immediate increase in *PS* without altering CBF or MABP. The time course and magnitude of this effect paralleled the expected changes in brain concentration of the drug. Shortly after injection of AMI, the brain concentration rises as the protein binding and lipid solubility characteristics of AMI favor distribution in lipid compartments (*6*); it then decreases rapidly as the drug is redistributed to the less vascularized peripheral adipose tissues. A single dose (7 mg/kg) produced a short-lived 22 percent increase in *PS* (more than 3 S.D.'s higher than the baseline value). The second injection (3.5 mg/kg) produced a 60 percent increase in *PS* consistent with a larger increase in brain concentration of the drug due to the loading effect of the initial dose. The third injection (3.5 mg/kg) caused a decrease in *PS*; in this case the brain concentration may have exceeded the maximum for producing this effect (*PS* increased approximately 30 minutes after the third injection, probably because of falling brain concentrations as the drug was redistributed). The decrease observed after starting CO_2 (shaded area) can also be related to concentration. AMI is weak base (pK_a = 9.4), which is normally highly protein-bound. Changing the *p*H of arterial blood from 7.4 to 7.0 by administering CO_2 should produce a 250 percent increase in free drug, which is the form in equilibrium with the adrenergic receptor. This increase should cause further receptor blockade and hence could be responsible for the reduction in *PS* observed with CO_2. Repeated AMI administration also caused a decrease in CBF (which is normally independent of systemic blood pressure over a wide range) even though MABP remained above autoregulatory limits (*4*). Both CBF and MABP increased after administration of angiotensin II at 108 minutes, yet CBF remained normally responsive to the addition of CO_2 to the inspired gas mixture at 83 minutes.

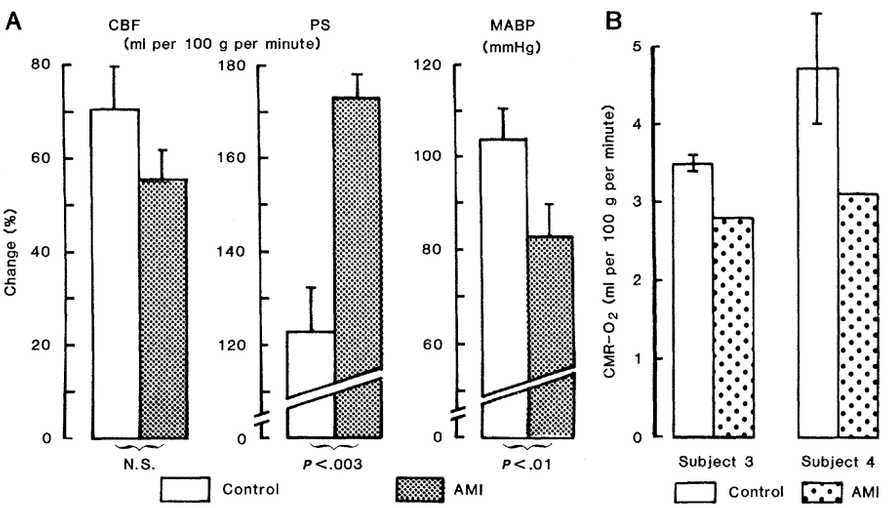

Fig. 2. (A) Changes in *PS*, CBF, and MABP produced by injection of AMI (7.5 mg/kg). (B) AMI-induced change in cerebral metabolic rate as measured by oxygen-15 (*5*) ($CMR-O_2$) in two of the monkeys. Three to five baseline measurements were made for each animal minutes before AMI administration. Sequential measurements were made as frequently as every 5 minutes for up to 2 hours. The differences between control and AMI values were analyzed by a two-tailed paired *t*-test. Values in (A) are the average (±1 S.D.) for all five animals. The control value in (B) is the average for three baseline measurements from each animal. Within 10 minutes, $CMR-O_2$ fell by more than 6 S.D.'s in subject 3 and by more than 2 in subject 4. In subject 3, it rose toward baseline for the next 20 minutes and then fell until the study was terminated 95 minutes after AMI administration, at which time $CMR-O_2$ was 2.48 ml per 100 g per minute. In subject 4, $CMR-O_2$ was low throughout the 60 minutes of observation and was 2.86 ml per 100 g per minute at termination. *N.S.*, not significant.

epinephrine from adrenergic fibers and by blocking its reuptake; at higher concentrations, AMI acts as an antagonist by blocking alpha-adrenergic receptors. The results do not establish the mechanism by which AMI alters *PS* in primates, but in rodents we showed that they are mediated by the central adrenergic system (*1*). The AMI-induced increase in the brain uptake of water is abolished either by pretreatment with 6-hydroxydopamine (intraventricularly) or phenoxybenzamine. The cerebrovascular effects of AMI are not mediated by the central serotonin, antimuscarine, or antihistamine actions of the drug.

AMI also induced a decrease in cerebral metabolic rate (Fig. 2), an observation supported by the report (*8*) that desipramine alters cerebral glucose utilization in rats at a dose and time which we have shown increase the brain uptake of water in rodents (*1*). Other investigators (*9*) reported that stimulation of the locus coeruleus and iontophoretic application of norepinephrine altered the responsivity of neurons in somatosensory cortex and lateral geniculate body to both excitatory and inhibitory afferent input, suggesting that the central adrenergic system may modulate transmission of information to these and possibly other neural systems.

All these findings are consistent with a system which acts to tonically influence cerebral function by regulating cerebral homeostasis. A system capable of adjusting the sensitivity of neurons to excitatory or inhibitory input could probably also affect cerebral oxygen utilization and the cerebromicrocirculation. Regulation of cerebromicrovascular permeability and cerebral metabolic rate is essential for normal cerebral functioning. The cerebral microenvironment must remain constant despite systemic changes in hydrostatic pressure and plasma osmolarity as well as changes in extracellular fluid within the brain due to regional metabolic shifts. Neural regulation would provide an additional means by which the brain can compensate for such changes. Substances whose passage across the blood-brain barrier may be similarly regulated could include electrolytes and metabolic substrates.

Whether these findings are relevant to the clinical use of antidepressants is not yet clear. However, the AMI-induced increase in *PS* occurs in primates as well as rodents and is observed in both species at concentrations which are therapeutic for human patients (*6*). Moreover, the central adrenergic system, which appears to mediate the *PS* effect of antidepressant drugs, is the neurotransmitter system thought to be involved in the pathophysiology of major mood disorders.

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Measurement of Intracellular Free Calcium in Monkey Kidney Cells with Aequorin

Abstract. *A method has been developed for the measurement of intracellular free calcium in mammalian cells. The calcium-sensitive photoprotein aequorin can be incorporated into isolated cells by hypo-osmotic treatment without altering the cell viability, permeability, or metabolism. Intracellular calcium activity (Ca_i^{2+}) was monitored in a perfusion system. In monkey kidney cells (LLC-MK$_2$), Ca_i^{2+} is approximately 57 nanomoles per liter. Changes in Ca_i^{2+} with time can also be followed: exposure of the cells to anaerobiosis or the calcium ionophore A23187 reversibly increases Ca_i^{2+}. The method has also been successfully tested in rat hepatocytes.*

Until now, it has been exceedingly difficult to measure cytosolic free calcium (Ca_i^{2+}) in mammalian cells. This difficulty has been a great obstacle in our understanding of the control, modulation, and regulation of cell calcium and of many calcium-dependent cellular functions (*1*). The calcium-sensitive photoprotein aequorin has been successfully injected into giant cells such as the squid axon and the barnacle muscle to measure Ca_i^{2+} (*2*). But the incorporation of aequorin or obelin, another calcium-sensitive photoprotein, into small mammalian cells has proved difficult or has been limited to virus-infected cell hybrids (*3*).

Table 1. Intracellular concentration of free calcium in cultured monkey kidney cells.

Experiment number	L (nA)	L_{max} (mA)	pF	Ca_i^{2+} (nM)
1	3.60	1.09	5.48	56
2	1.70	1.74	6.01	16
3	0.80	0.20	5.40	62
4	0.84	0.64	5.88	28
5	2.0	1.64	5.91	25
6	8.5	2.2	5.40	62
7	7.5	1.18	5.20	76
8	0.52	0.08	5.19	80
9	5.50	0.101	5.26	75
10	0.53	0.05	4.98	90
Mean ± standard error				57.0 ± 8.0

Aequorin is well suited to measure Ca_i^{2+} since its interaction with calcium is fast enough (time constant, 5 milliseconds) to reflect changes in Ca_i^{2+}. It is sensitive to Ca_i^{2+} and much less sensitive to intracellular free magnesium (Mg_i^{2+}), and it does not appear to bind to intracellular structures or alter intracellular functions (*4*). We have developed a method which incorporates enough aequorin to permit the detection of a calcium-dependent signal in suspensions of small mammalian cells and yet leaves the cells demonstrably intact and viable. We call this method the hypo-osmotic shock treatment (HOST).

Cultured monkey kidney cells (LLC-MK$_2$), grown as monolayers in Eagle's

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