studies were to demonstrate proportional differences between pongid and hominid species in the incidence of minor configurations (patterns 1 and 2), only the component of such differences that is independent of size (if any such component exists) would be useful in phylogenetic deduction. The occurrence by itself of a prismatic keyhole pattern in *Ramapithecus* suggests no closer kinship of that taxon to *H. sapiens* than to the extant apes.

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8. Abbreviations used in identifying teeth: R, right; M, molar; L, left; and P, premolar. Numerical subscripts and superscripts give the position of the tooth and whether it is an upper (superscript) or lower (subscript) one. Thus, RM^2 is the second right upper molar.
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11. We thank C. Frick of the Geological Survey of South Africa and M. J. Whitcomb of the University of the Witwatersrand for allowing us access to scanning electron microscopes, and S. C. Kammeyer and D. C. Panagos for technical assistance. Discussions with and comments from L. M. Jonck, P. Cleaton-Jones, P. V. Tobias, and C. K. Brain are gratefully acknowledged.

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Rapid Changes in Brain Benzodiazepine Receptors After Experimental Seizures

Abstract. *Seizures induced in the rat by electroshock or by injections of pentylenetetrazol increase the specific binding of diazepam to putative receptor sites in cerebral cortical membranes. The enhancement of diazepam binding results from a rapid increase in the number of available binding sites rather than a change in receptor affinity. The postictal increase in cortical benzodiazepine receptors suggests that the cerebral cortex might be more sensitive to the anticonvulsant effects of the benzodiazepines after seizures. This observation may be related to the mechanism of action of these drugs in the treatment of recurrent seizures such as status epilepticus.*

Diazepam and other closely related benzodiazepines are potent anticonvulsants in a wide variety of experimental and clinical seizure disorders; they are especially effective in the treatment of recurrent multiple seizures such as status epilepticus (*1*). Despite the unequivocal efficacy of the benzodiazepines in elevating seizure threshold (*2*) and rapidly inhibiting the spread of epileptic discharges from neocortical and rhinencephalic foci (*1, 3*), little is known about their underlying mechanism of action. Recent reports have demonstrated the presence of saturable, stereospecific, high-affinity diazepam-binding sites in the central nervous system of both rat and man (*4, 5*). Competition for this binding site by other benzodiazepines closely parallels their potency as anticonvulsants (*4, 5*) suggesting that these sites may function as receptors mediating the pharmacological actions of these drugs. If benzodiazepine receptors or their presumed endogenous ligands are related to the anticonvulsant actions of these compounds (*4*), they may normally be involved in the regulation or pathogenesis of seizure activity. We now report that both electrically and chemically induced seizures result in a rapid increase in the number of cortical benzodiazepine receptors without altering the apparent affinity of diazepam for these receptor sites.

Adult (125 to 150 g) male Sprague-Dawley rats (Taconic Farms) housed under standard laboratory conditions, were used in all experiments. Maximum electroshock seizures were induced with a Medicraft electroconvulsive therapy unit (150 V, 1 second, a-c) through ear clips attached to the pinnae (*6*). Generalized seizures, characterized by tonic-clonic movements, lasted for less than 1 minute. Subconvulsive electroshock (70 V, 0.4 second, a-c) was administered in the same manner. Control rats were subjected to the same procedures except that current was not applied. Chemically induced seizures were elicited by a single intraperitoneal injection of pentylenetetrazol (K & K Laboratories) (45 mg/kg in 0.9 percent saline). Control animals received saline alone. Only animals displaying generalized tonic-clonic movements within 2 to 3 minutes of injection were studied. Pentylenetetrazol-treated animals occasionally had free-running or multiple generalized seizures, or both. In order to control for the effects of interictal or postictal hypoxia on diazepam binding, additional rats were rendered hypoxic with argon gas (*7*). No seizures were observed in this group. Rats were killed by decapitation and crude synaptosomal (the second pellet) fractions of cerebral cortex (pooled frontal, temporal, and occipital cortices) were prepared as described (*5*). The final pellet was resuspended in 40 to 50 volumes of cold tris buffer (Calbiochem) (0.05*M*, *p*H 7.4) to a final protein concentration of approximately 1 mg/ml. Total, specific, and nonspecific diazepam binding was measured as described (*5*) with minor modifications (*8*). Specific binding refers to the total binding of [^{3}H]diazepam minus nonspecific binding which was obtained in the presence of 3 μM diazepam. Nonspecific binding was generally less than 5 percent of total binding at concentrations near the apparent dissociation constant (K_d) for diazepam. Nonspecific binding was not significantly affected by any of the experimental conditions, nor did pentylenetetrazol have any effect on diazepam binding in vitro at concentrations up to 1 μM (unpublished observations).

The amount of [^{3}H]diazepam specifically bound to cerebral cortical membranes (crude synaptosomal fraction) increased after seizures had been induced in the rats by electroshock; the binding increased by 21.2 percent ($P < .005$) and 21.4 percent ($P < .001$) at 15 and 30 minutes, respectively (Fig. 1). The binding of [^{3}H]diazepam returned to preseizure levels by 60 minutes. To determine if the changes in cortical diazepam binding were secondary to seizure-induced hypoxia, we investigated the effect of hypoxia on cortical benzodiazepine recep-

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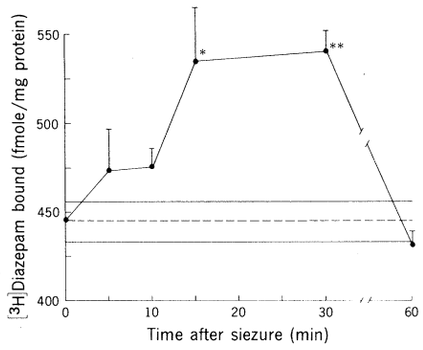

Fig. 1. Temporal changes in [^{3}H]diazepam binding in rats subjected to electrically induced seizures. Values represent means (± standard error) of four animals per time point. *$P < .005$; **$P < .001$ compared with sham-shocked controls. The binding of [^{3}H]diazepam to synaptosomal membranes from sham-shocked rats was 446 ± 12 fmole per milligram of protein ($N = 8$) at a diazepam concentration of 1.87 nM. The dashed line at the bottom of the figure represents the mean binding of sham-shocked controls; solid lines represent ± standard error. Control animals were killed from 5 to 60 minutes after a sham shock.

tors. No significant difference in diazepam binding was observed between control rats and rats rendered hypoxic by inhalation of argon gas (Table 1). In addition, subconvulsive electroshock was without effect on the number of cortical benzodiazepine receptors, indicating that a generalized seizure was a requisite for the observed changes (Table 1).

To determine whether enhanced diazepam binding results from an increased number of binding sites or from a change in receptor site affinity, we performed a Scatchard analysis on data derived from both electroshock and sham-shocked controls. Thirty minutes after seizures were induced by electroshock, the apparent K_d for [^{3}H]diazepam binding to cortical synaptosomes was not significantly different from that of sham-shocked controls ($K_d = 3.55 \pm 0.17$ nM as opposed to 3.36 ± 0.18 nM, respectively), while the total number of binding sites (B_{max}) increased by 24.7 percent (1779 ± 59 fmole per milligram of protein as opposed to 1427 ± 105 fmole, $P < .05$) (Fig. 2). A comparable increase (21.2 percent, $P < .05$) in the number of cortical diazepam-binding sites was also observed 30 minutes after seizures were induced by pentylenetetrazol, suggesting that the postictal enhancement of diazepam binding may be a general phenomenon (Table 1).

A number of explanations are compatible with a rapid increase in the number of diazepam-binding sites without an accompanying change in receptor affinity after seizures are induced experimentally. These include (i) an alteration in receptor turnover, (ii) a conformational change in the binding site, or (iii) a dissociation of an endogenous ligand from the binding site.

The rapid changes in cortical benzodiazepine receptors after the induction of generalized seizures are of the same order of magnitude as those reported for other central neurotransmitter receptors altered by various pharmacological manipulations (*9*). However, the rapid temporal changes in diazepam binding observed after seizures is in marked contrast to the changes observed in binding of other central neurotransmitter ligands (*9*) because only minutes, rather than days or weeks, were necessary for their development. The rapid increase in diazepam binding after seizures is similar to the rapid changes in the binding of catecholamine-receptor ligands in a number of peripheral systems (*10*). Since an increased number of binding sites in both the peripheral and central nervous system is usually associated with a heightened or supersensitive physiological response (*9, 10*), and the anticonvulsant activity of the benzodiazepines results from their marked inhibitory action on neuronal activity (*1, 3*), the increase in diazepam receptors observed after experimental seizures may enhance the physiological effect of a normally occurring inhibitory ligand. Nevertheless, the

Table 1. Effects of electrically or chemically induced seizures and hypoxia on [^{3}H]-diazepam binding. Animals were killed 30 minutes after treatment. The [^{3}H]diazepam binding to cerebral cortical membranes was measured as described (*5, 8*). Values represent means (± standard error) for four to eight animals per group. Statistical evaluations were made with Student's t-test. The concentrations of [^{3}H]diazepam used in these experiments were: Experiments 1 and 2, 1.8 nM; experiment 3, 2.8 nM; and experiment 4, 2.9 nM (*12*). NS, not significant.

Treatment	[^{3}H]Diazepam bound (fmole/mg protein)	P
	Experiment 1	
Electroshock	540 ± 12	<.001
Sham-shocked	446 ± 12	
	Experiment 2	
Pentylenetetrazol*	553 ± 16	<.05
Vehicle	456 ± 28	
	Experiment 3	
Electroshock†	573 ± 5	NS
Sham-shocked	613 ± 31	
	Experiment 4	
Argon	567 ± 22	NS
Air	572 ± 24	

*The dose was 45 mg/kg, injected intraperitoneally. †Subconvulsive.

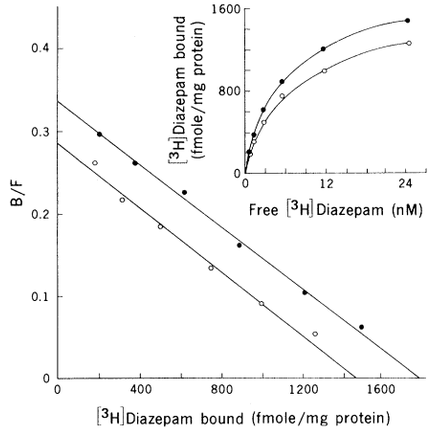

Fig. 2. Kinetics of [^{3}H]diazepam binding in rats subjected to electroshock and sham shock. The inset shows the saturation isotherm and [^{3}H]diazepam binding as a function of increasing concentrations of [^{3}H]diazepam. Open circles, sham-shocked control rats; solid circles, rats subjected to electroshock. B/F, ratio of bound to free [^{3}H]diazepam.

data presented here suggest that the postictal cerebral cortex may be altered with respect to its pharmacologic response to benzodiazepines. These findings may, therefore, be relevant to the marked potency and rapid therapeutic effects of these drugs in the treatment of recurrent seizure disorders such as status epilepticus (*1, 11*).

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Thirty minutes after anoxia was induced (when the maximum binding of [^{3}H]diazepam was observed after electrically or chemically induced seizures), the animals were killed for determination of cortical benzodiazepine receptors.

8. Specific [^{3}H]diazepam binding was determined as described (*5*) with the following minor modifications. Synaptosomal suspensions were incubated in a total volume of 1.5 ml. Each preparation consisted of 1.0 ml of tissue suspension (crude synaptosomal fraction), 0.4 ml of tris-HCl buffer (0.05*M*, *p*H 7.4), 0.0375 ml of distilled water or nonradioactive diazepam (3 μM), and [^{3}H]diazepam (specific activity, 39.08 Ci/mmole, New England Nuclear) diluted to the appropriate concentration with distilled water. The preparations were incubated for 15 minutes in an ice bath at 0° to 4°C. Incubation was terminated by filtering through Whatman GF/B filters and washing the filter with 10 ml of ice-cold tris-HCl buffer (0.05 *M*, *p*H 7.4). Filters were suspended in 10 ml of Aquasol (New England Nuclear) and the radioactivity measured with a Beckmann LS-355 liquid scintillation counter.
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11. Diazepam is recommended as the drug of choice in the treatment of status epilepticus (*1*). A dose of 2 to 20 mg administered intravenously is effective in most cases. This compares to dosages of 200 to 400 mg and 300 to 500 mg for phenobarbital and diphenylhydantoin, respectively. Diazepam is 50 to 100 times more potent than the other commonly used anticonvulsants in this disorder [F. A. Elliott, *Clinical Neurology*, (Saunders, Philadelphia, 1971), pp. 140–141.]
12. Scatchard and saturation analyses (see Fig. 1) indicate seizure-induced alterations in receptor number rather than affinity. This enabled us to use a range of ligand concentrations in these experiments, since the relative changes in receptor number observed would be independent of the concentration used.
13. S.M.P. is a research associate in the Pharmacology Research Associate Training Program, National Institute of General Medical Sciences. P.S. was a guest worker at the Laboratory of Chemistry, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, during these studies.

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The Red Cell as a Fluid Droplet: Tank Tread-Like Motion of the Human Erythrocyte Membrane in Shear Flow

Abstract. *When whole human blood is subjected to viscometric flow, individual red cells are seen to be elongated and oriented in the shear field. In addition, a tank tread-like motion of the membrane around the cell content occurs. In dilute suspensions of erythrocytes in viscous media, the same behavior is better observed and can also be measured quantitatively.*

The peculiar shape and unusual deformability of mammalian red blood cells (RBC's) have been the subject of much scientific debate. In the last century conflicting concepts of the structure of the red cell were advocated; hypotheses ranged from the assumption of an elastic internal matrix to the model of a flexible membrane shell filled with a fluid (*1*). In the first part of this century the low viscosity of RBC suspensions led to the model of blood as an emulsion (*2*). This idea was taken up by Dintenfass (*3*), who stated that the fluidity of concentrated RBC suspensions can only be explained if the cytoplasm of the red cell is liquid and participates in shear flow. By measuring the viscosity of RBC suspensions in which the viscosity of the suspending phase and the volume concentration of RBC's were varied and comparing the results with a theoretical equation for the viscosity of concentrated emulsions, he estimated the viscosity of the cytoplasm (*4*). Furthermore, by using a theory for the viscosity of emulsions, he postulated a membrane that becomes an extremely inviscid liquid surface film when the RBC suspension is sheared (*4*). Alternatively, Schmid-Schönbein and Wells (*5*) explained the viscometric data in terms of a tank tread-like motion of the whole membrane around the cell content. This concept was based on microscopic observation of RBC suspensions under shear. Füredi and Ohad (*6*) had reported a similar membrane motion of RBC's subjected to an alternating electromagnetic field. We present here an improved technical approach and a closer analysis of this phenomenon.

A suspension of RBC's was placed in a counterrotating transparent cone-and-plate chamber (Fig. 1) adapted to an inverted interference contrast microscope (*7*). When focusing the stationary layer of the shear field, it was possible to observe RBC's under shear without translational movement. Cinematographic records were taken with a high-speed camera driven to a maximum of 500 frames per second (*8*) and synchronized to strobe illumination (*9*). The cone angle was 1.5°. Observations were made at distances of 0.5 to 1.5 mm from the axis of revolution. The observed shear rate ($\dot{\gamma}$) for each experiment was obtained from the difference between the peripheral speeds of cone and plate divided by their distance. Erythrocytes were suspended either in autologous plasma or in viscous solutions of dextran (*10*) in phosphate-buffered saline. Dextran solutions were adjusted to isotonicity within a 10 percent error by measuring the vapor pressure (*11*). The viscosities of the dextran solutions (η_0) were measured with a capillary viscometer (*12*) and corrected for the temperature of the room in which the experiments were done. To prevent crenation, human albumin (0.1 to 0.3 g per 100 ml) was added. Latex particles (diameter, 0.8 μm) (*13*) were used as external membrane markers, but only in experiments with dextran. Heinz bodies (hemoglobin precipitates), produced by incubation of RBC's (hematocrit, 2.5 percent) in acetylphenylhydrazine (2 mg/ml) in phosphate-buffered saline at 30°C for 4 hours, were used as internal membrane markers and also as markers of the cytoplasm.

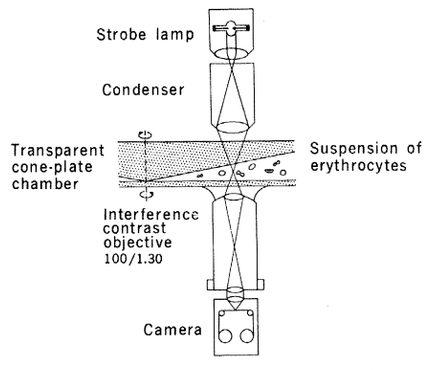

Fig. 1. Schematic drawing of the setup (for details see text).

When whole human blood—that is, red cells in plasma (hematocrit, 40 to 45 percent)—is subjected to shear rates above 500 sec^{-1} the RBC's are elongated flat ellipsoidal bodies (*14*), which are irregular in shape and not stationary. When one observes an individual cell, however, it becomes evident that for most of the time the main axis of the ellipsoid is oriented approximately parallel to the direction of flow and that its flat faces are parallel to the planes of shear. When $\dot{\gamma}$ or the hematocrit or both are elevated, the cells become more elongated, assume a more stationary orientation, and show less change of shape. In the oriented state of the RBC, the tank tread motion of the membrane can be observed through the Heinz bodies bound to the cytoplasmic side of the membrane.

To simplify the fluid mechanical boundary conditions of an individual cell, RBC's were suspended at a low hematocrit in viscous solutions. In these suspensions, the behavior mentioned above is not complicated by cell-cell interactions and is therefore more regular. Figure 2, A and B, shows a resting biconcave red cell. In Fig. 2C the same cell is elongated and oriented in the shear field.

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