volvement of the AVC as indicated by its metabolic response (Fig. 2).

The combination of positron computed tomography to measure the concentration of radioactivity in tissue in vivo, together with the use of physiologically active compounds labeled with positron-emitting isotopes (for example, ^{11}C, ^{13}N, ^{15}O, and ^{18}F) (*19*) and tracer kinetic models allows one to measure local physiological processes in human subjects. By selecting appropriate labeled compounds and tracer kinetic models, studies of local neurotransmitters, membrane transport, blood flow, protein synthesis, and other physiologic processes are feasible.

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19. Fluorine-18 is used as a hydrogen or hydroyl replacement because of its similar size (for example, similar steric hindrance), strong carbon-fluorine bond, and minimum effects of the differences in electronegativity. Whereas ^{18}F is used to label analogs of natural compounds, ^{11}C, ^{13}N, and ^{15}O are used as direct substitutes of these elements in natural compounds.
20. Partially supported by Department of Energy grant DEAMOS-76-SF00012 and NIH grants ROI-GM-24839-01 and PO-NS 156540-01.

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Pentobarbital: Dual Actions to Increase Brain Benzodiazepine Receptor Affinity

Abstract. *The binding of [^{3}H]diazepam to benzodiazepine receptors was studied in extensively washed membranes of rat cerebral cortex in the presence of the depressant barbiturate, pentobarbital. Pentobarbital, like the endogenous neurotransmitter γ-aminobutyric acid (GABA), increased the basal binding and also potentiated the GABA-enhanced binding of [^{3}H]diazepam to benzodiazepine receptors by increasing the apparent affinity of [^{3}H]diazepam for the benzodiazepine receptor. The concentrations of pentobarbital necessary to elicit these effects in vitro are the same as those observed after treatment with pharmacologically relevant doses, suggesting that a common neurochemical association may exist between these two types of compounds.*

Barbiturates are effective general anesthetics, sedative-hypnotics, and anticonvulsants (*1*) and share the last two therapeutic effects with the benzodiazepines. There is also compelling electrophysiological evidence to suggest that both barbiturates (*2*) and benzodiazepines (*3*) enhance chloride-dependent synaptic events mediated by γ-aminobutyric acid (GABA), a widely distributed transmitter in the central nervous system (CNS) with well-established inhibitory functions (*4*). In addition, anesthetic and sedative-hypnotic barbiturates can, like GABA, directly inhibit excitability by increasing chloride conductance; this effect is blocked by drugs that antagonize the inhibitory actions of GABA (*2*, *3*).

Benzodiazepines are believed to elicit their pharmacologic effects by interacting with a specific type of receptor that is highly concentrated in the CNS (*5*). This receptor appears to be functionally coupled to both a GABA receptor and a

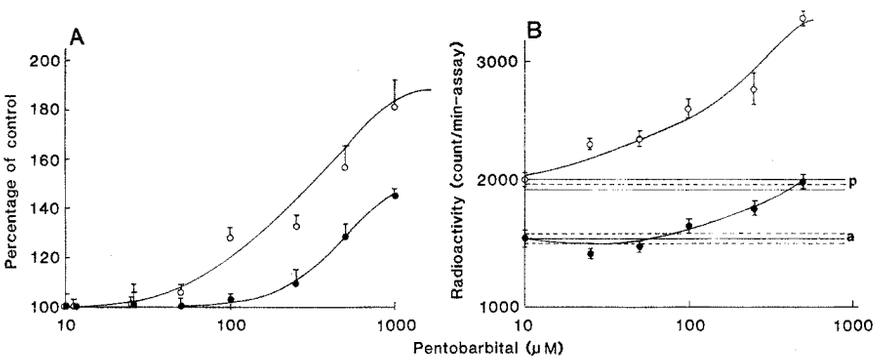

Fig. 1. Effects of pentobarbital on [^{3}H]diazepam binding to benzodiazepine receptors. (A) Pentobarbital enhancement of [^{3}H]diazepam binding in 50 m*M* tris HCl buffer (*p*H 7.4) (○) and 50 mM tris-maleate buffer (*p*H 7.4) (●). Values represent the mean ± standard error of three to eight experiments in well-washed membranes of rat cerebral cortex prepared as described in the text. The specific binding (*8*) of [^{3}H]diazepam was determined at a radioactive ligand concentration of 0.3 n*M*. The mixtures incubated contained 0.4 to 0.6 mg of protein per assay. (B) Potentiation of GABA-enhanced [^{3}H]diazepam binding by pentobarbital. Values represent the means ± standard error of triplicate determinations from a representative experiment. Symbols: [^{3}H]diazepam binding in the absence, *a*, or presence, *p*, of 0.1 μ*M* GABA; (●), [^{3}H]diazepam binding in the presence of pentobarbital (200 μ*M*) or (○) pentobarbital (200 μM) plus GABA (0.1 μ*M*). This experiment was done in tris-maleate buffer. The experiment was repeated twice with similar results and was also repeated in tris-HCl buffer, which resulted in an absolute increase in the [^{3}H]binding both in the presence and absence of the drugs. The enhanced binding in the presence of tris-HCl buffer is a result of a significant reduction in the apparent affinity of [^{3}H]diazepam (*6*, *7*). In paired experiments in which 0.3 n*M* [^{3}H]diazepam was used as a radioactive ligand, incubation of membranes in tris-maleate buffer resulted in a 50 percent reduction in basal binding (*6*, *7*, *9*).

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chloride ionophore, since both GABA and anions (such as chloride, bromide, and iodide) increase the affinity of this receptor for benzodiazepines (*6, 7*). Despite the many common pharmacologic and neurophysiologic properties shared by the barbiturates and benzodiazepines, previous attempts to demonstrate a common neurochemical link between these two compounds have been unsuccessful.

We now report that pentobarbital, which potentiates GABA-mediated activation of chloride ion conductance [and at higher concentrations, activates chloride conductance alone (*2, 3*)], stimulates basal [^{3}H]diazepam binding and markedly potentiates GABA-enhanced [^{3}H]diazepam binding in well-washed cerebral cortical membranes. The concentrations of pentobarbital required to increase basal binding of [^{3}H]diazepam in vitro correspond to the anesthetic levels of pentobarbital achieved in vivo, whereas the concentrations required to potentiate the effects of GABA on the benzodiazepine receptor occur at subanesthetic (that is, sedative-hypnotic) concentrations. These changes in [^{3}H]diazepam binding result from an increased affinity of the benzodiazepine receptor for diazepam rather than an alteration in receptor number.

Adult (175 to 200 g) male Sprague-Dawley rats (Taconic Farms, Germantown, New York) housed under standard laboratory conditions, were used in all experiments. The rats were killed by decapitation, the cerebral cortex (pooled parietal, occipital, temporal, and frontal cortices) was removed, disrupted in 100 volumes of buffer (either tris-maleate or tris-HCl, 50 m*M*, *p*H 7.4) with a Brinkmann Polytron (setting 6, 15 seconds), and then centrifuged at 20,000*g* for 20 minutes. The resulting pellet was washed twice in 100 volumes of buffer, resuspended in the original volume of buffer, and fast-frozen on solid CO_2 for at least 12 hours before being assayed. The tissue was defrosted, recentrifuged, and resuspended immediately prior to assay. Binding of [^{3}H]diazepam was carried out in a total incubation volume of 1.5 ml as previously described (*8*).

Sodium pentobarbital (Abbott) stimulated [^{3}H]diazepam binding in both chloride-free and chloride-enriched buffer (Fig. 1, A and B). However, both the maximum enhancement by pentobarbital in the chloride-free (46 percent) medium and the EC_{50} (concentration necessary to elicit a half-maximum effect) (400 μ*M*) were significantly enhanced in the presence of 50 m*M* chloride (81 percent and 220 μ*M*, respectively). In agreement with a previous report (*6*) basal binding of [^{3}H]diazepam was significantly lower in the chloride-free medium; the chloride ion concentration required (10 m*M*) to restore the stimulatory effects of sodium pentobarbital was similar to that necessary to restore basal levels of [^{3}H]diazepam binding to the membranes (*6, 7, 9*). The increases in [^{3}H]diazepam binding elicited by pentobarbital (1 m*M*) were antagonized by the GABA antagonist bicuculline and the chloride-ionophore blocking agent picrotoxin, with IC_{50} values (concentrations that inhibit binding by 50 percent) of 1.2 and 87 μ*M*, respectively, in tris-HCl buffer (*10*).

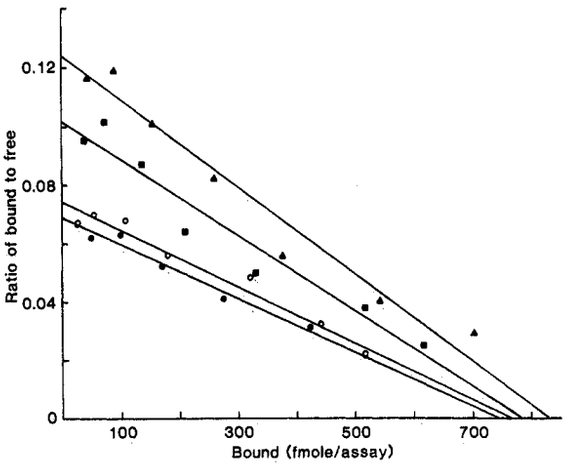

Fig. 2. A representative Scatchard analysis of binding in the presence of tris-maleate buffer, with concentrations of [^{3}H]diazepam ranging from 0.3 to 20 n*M*. Symbols: (●), basal; (○), sodium pentobarbital (200 μ*M*); (■), GABA (0.1 μ*M*); and (▲), GABA (0.1 μ*M*) plus pentobarbital (200 μ*M*). In this representative experiment, the K_d values were 7.2, 6.9, 5.2, and 4.5 n*M*, respectively, for basal, pentobarbital, GABA, and GABA plus pentobarbital. The correlation coefficient (*r*) was greater than .97 for each of these lines. The mixtures incubated contained 0.4 mg of protein per assay. This experiment was repeated three times in both tris-maleate and tris-HCl with similar results (*13*).

In chloride-free medium, the response to submaximum (0.1 μ*M*) concentrations of GABA was signicantly potentiated by pentobarbital at concentrations as low as 25 μ*M* (Fig. 1B). The latter concentration is tenfold lower than concentrations that produced only a modest enhancement (10 percent) in [^{3}H]diazepam binding in the absence of GABA (Fig. 1, A and B). The pentobarbital-stimulated increase in [^{3}H]diazepam binding was due to an increase in receptor affinity, as has been reported for GABA-stimulated enhancement of benzodiazepine binding (*11*) (Fig. 2). The potentiation of GABA-enhanced [^{3}H]diazepam binding was observed in both chloride-free and chloride-enriched buffer (Fig. 2) (*12*). However, subsequent studies were performed in chloride-free buffer because the attenuated response to pentobarbital was in this medium. In the representative Scatchard plot (Fig. 2), a submaximum concentration of GABA potentiated diazepam binding by increasing the apparent affinity of the radioactive ligand. Pentobarbital (200 μ*M*) alone had only a marginal effect on the apparent affinity of [^{3}H]diazepam (Fig. 2) (*13*). However, a combination of GABA and pentobarbital caused a statistically significant potentiation ($P < .02$) of GABA-enhanced [^{3}H]diazepam binding (*13*). Maximum stimulatory concentrations of GABA (for example, 30 μ*M*) were not potentiated by pentobarbital; at these concentrations of GABA, effects of the two agents become nearly additive (*12*).

The results reported here demonstrate that pentobarbital enhances the apparent affinity of diazepam for the benzodiazepine receptor in two ways: (i) directly (with an EC_{50} of about 200 μ*M* in chloride-containing medium) and (ii) indirectly by potentiating the stimulatory effects of GABA (*14*). These effects were dependent on the inclusion of chloride and antagonized by pharmacologic concentrations of either bicuculline or picrotoxin (*10*). Electrophysiological analysis of the effects of pentobarbital on GABA-activated chloride conductance revealed that pentobarbital can potentiate the activation of chloride ion conductance by GABA at tenfold lower concentrations than are required for direct activation of chloride ion conductance (*2, 3*). Despite the concordance of both neurochemical and electrophysiological observations, it is unclear if the dual actions of pentobarbital on [^{3}H]diazepam binding are the result of an action at one or more sites. The observation that both bicuculline and picrotoxin can reverse pentobarbital-enhanced [^{3}H]diazepam binding may reflect a close association of the benzodiazepine receptor with both a GABA receptor and a chloride ionophore. Furthermore, the presence of an endogenous inhibitor or inhibitors of GABA-enhanced benzodiazepine binding (*15*) suggests that naturally occurring antagonists of pentobarbital's depressant effects may also exist.

Thus, the direct and indirect activation

of chloride ion conductance by pentobarbital closely parallels its dual stimulatory actions on diazepam binding in vitro. These results suggest a close functional relation between chloride conductance mechanisms that are activated by both GABA and pentobarbital and regulation of the benzodiazepine receptor. The proposed functional relation between pentobarbital and the GABA–benzodiazepine–chloride ionophore receptor complex may provide a neurochemical basis for the pharmacologic effects shared by benzodiazepines and barbiturates.

Note added in proof: After submission of this manuscript, a report appeared confirming the enhancement of benzodiazepine binding by pentobarbital (*16*).

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10. Similar IC_{50} values (1.8 and 75 μM) were obtained for bicuculline and picrotoxin, respectively, in tris-maleate buffer (S. Paul, P. Marangos, P. Skolnick, in preparation).
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13. Statistical analysis revealed a significant reduction ($P < .02$) in the K_d of [^{3}H]diazepam with GABA plus pentobarbital compared to GABA alone. Pentobarbital (200 μM) did not elicit a statistically significant reduction in the K_d of [^{3}H]diazepam ($P > .1$) in tris-maleate buffer. A statistically significant difference ($P < .05$, paired t-test) was obtained by comparing the algebraic sum of the decrease in K_d obtained with GABA and pentobarbital alone with the decrease in K_d obtained with a combination of these agents. That is, the difference between [(GABA − basal) + (pentobarbital − basal)] was significantly different from [(pentobarbital + GABA) − basal]. No statistically significant differences in B_{max} were obtained in an identical analysis.
14. In agreement with our findings, C. Braestrup and R. S. Squires [*Eur. J. Pharmacol.* **48**, 263 (1978)] previously reported that they found no significant effect of 3 μM pentobarbital on [^{3}H]diazepam binding. In another report, C. Mackerer *et al.* [*J. Pharmacol. Exp. Therap.* **206**, 405 (1978)] reported no significant inhibition of [^{3}H]diazepam binding with 100 μM pentobarbital in the supernatant fraction from a homogenate centrifuged at 600*g*. Such a preparation contains high (> 30 μM) concentrations of endogenous GABA, which would mask the potentiation of submaximum concentrations of GABA reported here.
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Noise Raises Blood Pressure Without Impairing Auditory Sensitivity

Abstract. *Two rhesus monkeys, exposed continuously to realistic patterns and levels of noise for 9 months, exhibited sustained elevations in blood pressure that did not return to baseline values after the noise ended. Auditory brainstem responses, measured before and after exposure, indicated no change in auditory sensitivity.*

There is general agreement that exposure to noise of sufficient intensity and duration can damage the inner ear (*1*). Despite an extensive literature (*2*), however, there is no such agreement that noise can produce other physiological effects. Considerable evidence accumulated over the past three decades from both human and animal studies (*3*) suggests, for example, that noise can impair blood pressure regulation, particularly in the direction of hypertension; yet research in this area has also generated contradictory results that seriously obscure the relationship (*4*). In view of the ambiguous association between noise and hypertension, it is not surprising that the derived association between noise-induced hearing loss and hypertension is ambiguous as well (*5*).

The purpose of our study was twofold: (i) to explore possible long-term blood pressure and other cardiovascular adjustments to noise and (ii) to determine whether or not decrements in auditory function might accompany such adjustments.

Several refinements not ordinarily found in research on noise effects were incorporated into our experimental design. For example, we specified and controlled the total noise exposure of our subjects; measured responses directly, automatically, and frequently, assuring a high level of data stability; minimized, or held within a narrow range, several variables known to elicit cardiovascular adjustments similar to those often ascribed to noise alone; and monitored auditory pathway function before and after noise exposure.

Four young adult female rhesus monkeys were used. Their average weight was 4.3 kg. We restrained all the animals

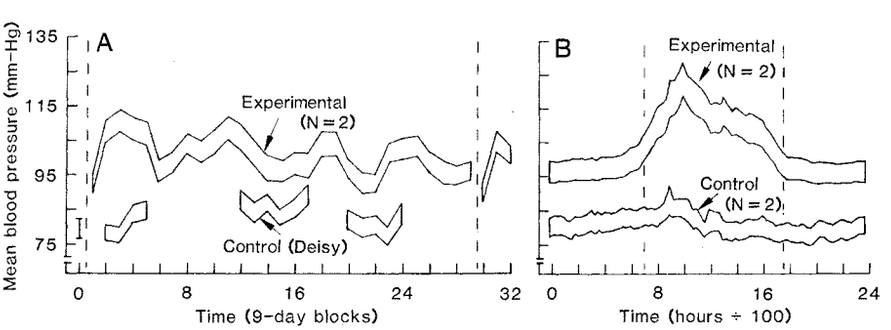

Fig. 1. Comparison of mean blood pressure for experimental and control animals. Envelopes encompass the mean and 95 percent confidence limits. (A) Overall trends. Dashed vertical lines indicate onset and cessation of noise exposure period. Thick vertical bar at left represents pre-exposure values for experimental animals. (B) Diurnal rhythm. Dashed vertical lines encompass times when experimental animals were exposed to the most intense noise episodes. Animals were fed between 0900 and 1100 and cleaned between 0900 and 1200.

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