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-benzodiazepinechloride 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). P. SKOLNICK

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 Statistical analysis revealed a significant reduction (P < .02) in the K_d of [³H]diazepam with GABA plus pentobarbital compared to GABA alone. Pentobarbital (200 μM) did not elicit a statistically significant reduction in the K_d of [³HIdiazepam (P > 1) in tris.maleate huffer A [³H]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 ageorate 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
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6 October 1980; revised 30 December 1980

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 noiseinduced 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 adiustments.

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 preexposure 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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in chairs within the experimental environment for 24 hours a day for 6 to 9 months before the experiment proper began. After this adjustment period, we surgically passed an open cannula into the abdominal aorta of each animal so that the orifice rested 1 to 2 cm below the renal arteries. The pressure-pulse waveforms arising from this site were transduced, conditioned, and digitally sampled at a rate of 200 Hz. The sampling algorithm devised for the DECLAB 11/ 03 computer, which controlled data acquisition and analysis, provided for continuous monitoring of each animal for 15 seconds of each minute. Including periods when no measurements were taken, experimental animals were sampled for about 19 percent of the entire time of the experiment. For each 1-minute period, heart rate and average systolic, areabased mean and diastolic blood pressure were computed and stored in temporary memory. Minute-by-minute noise levels, in terms of the A-weighted average energy equivalent level $[L_{Eq}dB(A)]$ (6), were also computed and stored at the same time. Every 20 minutes, the mean (\bar{X}) and standard deviation of the 20 1-minute samples for each animal and each variable were computed and stored on disk for off-line analyses. Daily estimates of each variable, therefore, were based on 1440 averages, each of which was, in turn, based on 3000 samples.

After surgery, we returned the animals to sound-attenuating booths. Experimental animals (N = 2) were maintained under low noise conditions only until blood pressure levels were stable for nine consecutive days. Control animals (N = 2) were maintained under similar conditions throughout their periods of confinement. Within the sound-attenuating booths, light, temperature, and humidity were maintained within narrow ranges while contaminants were minimized through the use of efficient ventilation and waste disposal.

Once stable baselines were established for them, the experimental animals were exposed continuously each day for 9 months to a recorded noise exposure sequence (NES), which consisted of six episodes presented at appropriate times (for humans) throughout the day. The resulting pattern of daily exposure was both complex and realistic. It was meant to resemble the daily pattern of noise to which a worker in a noisy industry might be subjected. The 24-hour L_{Eq} was set to register 85 dB-Fast and 97 dB-Peak on our sound level meter (General Radio 1982). We had previously found that such levels avoid short-term (1 to 2 weeks) acoustic trauma in rhesus mon-



Fig. 2. Latency of auditory brainstem response components P_1 and $P_{4,5}$ for the two experimental animals, Magnolia (triangles) and Chorreada (squares), measured before (open symbols) and after (filled symbols) 9 months of exposure to noise. Envelopes represent 95 percent confidence intervals for component latencies derived from normative data (8).

keys as measured by evoked response techniques. To ascertain possible changes in auditory funcion associated with long-term noise exposure, however, the latencies of click-evoked, auditory brainstem responses (ABR) (7) were measured in the two experimental animals shortly before onset and after cessation of the 9-month exposure period. In addition, we continued to monitor cardiovascular responses for 27 days after the noise ended.

During their low-noise confinement, mean blood pressure for the two control animals, Deisy and Pupuchita, were 82.8 and 79.1 mm-Hg, respectively ($\bar{X} = 81.8$ mm-Hg). During the low-noise portion of their confinement (pre-exposure period), mean blood pressure levels of the two experimental animals, Magnolia and Chorreada, were 73.3 and 84.5 mm-Hg, respectively ($\bar{X} = 78.9$ mm-Hg), an average difference of only 3.5 percent between experimentals and controls.

Both experimental animals reacted to long-term noise exposure with sustained elevations of blood pressure. Mean blood pressure rose to 103.3 mm-Hg for Magnolia and 97.7 mm-Hg for Chorreada $(\bar{X} = 100.5 \text{ mm-Hg})$ during the exposure period. Compared with controls, the net increase in blood pressure for the experimental animals was 18.7 mm-Hg or 22.9 percent.

A comparison of intrasubject responses before and during the noise revealed increases of 41.8 percent for Magnolia and 15.6 percent for Chorreada $(\bar{X} = 27.0 \text{ percent})$. Since mean blood pressure was similar for the two animals over the pre-exposure period, this substantial difference was chiefly related to Magnolia's relatively low mean blood pressure in the pre-exposure period.

Averaged trends of mean blood pressure over the course of the experiment are shown in Fig. 1A. Blood pressure of the control animal shown, Deisy, was measured intermittently over the course of 7.5 months. There was no overlap in the averaged data of the two experimental animals when compared with this one control animal. Blood pressure remained elevated for the 27 days that we monitored responses after the noise was turned off (Fig. 1A).

In small-N research, one of the most convincing demonstrations of a causal relationship between independent and dependent variables arises when the systematic manipulation of the former produces corresponding changes in the latter (8). In our study, manipulation of the independent variable chiefly took the form of repeated variation of noise exposure intensity. Corresponding changes in the dependent variable, mean blood pressure, buttresses the argument for a cause-effect relationship between the two variables.

The diurnal rhythm of mean blood pressure for the experimental animals during continuous noise exposure differed noticeably both from the rhythm they displayed before noise (not shown) and from the diurnal rhythm displayed by the two control animals (Fig. 1B). Blood pressure of the experimental animals was especially elevated during the interval corresponding to the presentation of the most intense noise episodes. The diurnal rhythm of noise level was highly correlated with that of mean blood pressure (r = .825, P < .001).

By the time we had begun this experiment, normative information regarding the latencies of click-evoked ABR's in rhesus monkeys was available (9). We used this information to determine the functioning of peripheral and subcortical auditory pathways in the two experimental animals. Latencies of ABR components P1 and P45 for a wide range of stimulus intensities were determined before and after 9 months of noise exposure. Each animal was compared with itself and with the normative data. Both animals displayed nearly average latencies prior to noise exposure (Fig. 2); we observed no reliable deviation from these latencies 13 to 15 hours after cessation of the most intense noise episode. This makes it highly unlikely that permanent noise-induced changes in auditory sensitivity occurred.

We have demonstrated for what we believe to be the first time in a carefully controlled experiment that moderate levels of realistic noise, presented at appropriate times throughout the day, can produce sustained elevations in blood pressure without producing significant changes in auditory sensitivity. While extrapolation from one species to another must always be undertaken with caution, we have provided evidence, based on a primate model, that these two categories of event may occur independently in humans exposed to moderately intense noise over long periods of time. Further, we have demonstrated that noise effects do not necessarily dissipate when the noise ends.

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- ABR is the acronym recommended by the joint U.S.A.-Japan Seminar on Auditory Responses from the Brainstem, Honolulu, Hawaii, 27 No-7. from the Brainstem, Honolulu, Hawaii, 27 No-vember to 1 December 1979 [See H. Davis, *Laryngoscope* 89, 1336 (1979)]. The latency of the ABR component has proved to be the most consistent and reliable diagnostic index for sub-cortical auditory pathway function.
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- 8 July 1980: revised 4 October 1980

An Endocrine Approach to the Control of Epidermal Growth: Serum-Free Cultivation of Human Keratinocytes

Abstract. Human keratinocytes, derived from the skin of newborns and of adults, were grown in the complete absence of serum, in a hormone-supplemented medium on fibronectin-coated cell culture dishes at low seed density. The cell culture medium consisted of Medium 199 containing epidermal growth factor, triiodothyronine, hydrocortisone, Cohn fraction IV, insulin, transferrin, bovine brain extract, and trace elements. Removal of the brain extract from the hormone supplement had a greater negative impact on proliferation of the keratinocyte cultures than did the removal of epidermal growth factor, hydrocortisone, and triiodothyronine or Cohn fraction IV. The growth of keratinocytes in this hormone-supplemented medium suggests that control of keratinocyte growth depends in large part on endocrine stimulation by other body organs, including the brain.

The serum dependence of mammalian cell growth in vitro is mediated by combinations of serum-derived hormones and growth factors (1). Certain fastidious cells incapable of growth in serum-supplemented medium require the use of feeder layers, cell-conditioned medium, very high seed densities, or organ extracts (2, 3) as possible sources of additional specific growth factors. Human keratinocytes have been successfully cultivated on lethally irradiated 3T3 cell feeder layers in the presence of serum (4), with cell growth being further improved by the addition of epidermal growth factor and hydrocortisone to the medium (5).

We recently demonstrated that a fibronectin matrix provides a biologically relevant surface for the attachment of human keratinocytes in vitro (6). The use of a fibronectin-coated surface permits keratinocyte growth at low seed density in the absence of a 3T3 cell feeder layer or other contaminating cell types (6), and at serum concentrations considerably below those apparently needed in other systems (4-7). This approach has enabled us to identify factors necessary for the serum-free cultivation of human keratinocytes and has resulted in an appreciation of the brain as an important source of keratinocyte growth-promoting activity.

Primary cultures of human keratinocytes were obtained from trypsin-split epidermal sheets of human skin specimens as previously described (5). Both neonatal foreskins and facial skin from adults aged 56 to 62 years were used in the experiments reported here. Singlecell suspensions were plated on cell culture dishes previously coated with purified human fibronectin (10 μ g/cm²) (7) at



Fig. 1. Human keratinocyte growth in a serum-free environment with and without the addition of 1 percent fetal bovine serum: A single cell suspension of human keratinocytes was plated at a seed density of 5×10^4 cells per square centimeter on cell culture dishes (35 mm, Falcon) coated with 10 µg of human fibronectin per square centimeter. The medium was replaced every 2 to 3 days with the appropriate medium. The hormone supplement (7 GF's) contained 10 ng of epidermal growth factor per milliliter (EGF, Collaborative Research, Waltham, Mass.), 10 µg of insulin per milliliter (I, Sigma), 10 µg of transferrin per milliliter (T, Sigma), 5 X 10^{-5} M hydrocortisone (HC, Sigma), 10^{-9} M triiodothyronine (T_3 , Sigma), 150 µg per milliliter of human Cohn fraction IV (CF_{IV} , Ar-

mour Pharmaceuticals, Chicago), 150 µg per milliliter of bovine brain extract (BE) and trace elements in Medium 199 (Gibco, Grand Island, New York). (A) (Left to right) 20 percent FBS control; seven growth factors minus EGF (-EGF); seven growth factors minus bovine brain extract (-BE); and hormone supplement control (7 GF's). (B) Seven growth factors plus 1 percent FBS minus human Cohn fraction IV $(-CF_{1V})$; seven growth factors plus 1 percent FBS minus triiodothyronine and hydrocortisone $(-HC, T_3)$; seven growth factors plus 1 percent FBS minus bovine brain extract (-BE); seven growth factors plus 1 percent FBS control. (C) Seven growth factors minus human Cohn fraction IV $(-CF_{IV})$; seven growth factors minus triiodothyronine and hydrocortisone $(-HC, T_3)$; seven growth factors minus bovine brain extract (-BE); and the hormone supplement control (7 GF's). After 15 days of growth, the cultures were fixed with 4 percent Formalin for 40 minutes and stained with 1 percent Rhodanile blue (MCB, Norwood, Ohio) for 1 hour.

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