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Cerebral Blood Flow and Cerebrovascular Permeability in an Inescapable Shock (Learned Helplessness) Animal Model of Depression[1]

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HUGHES, C. W., T. A. KENT, J. CAMPBELL, A. OKE, H. CROSKELL AND S. H. PRESKORN. *Cerebral blood flow and cerebrovascular permeability in an inescapable shock (learned helplessness) animal model of depression.* PHARMACOL BIOCHEM BEHAV 21(6)891–894, 1984.—The effects of a purported animal model of depression (inescapable shock, IS) was tested on: (a) escape behavior, (b) regional brain levels of norepinephrine (NE), serotonin (5-HT), and dopamine, and (c), the response of the cerebromicrovasculature to metabolic demand as mimicked by manipulation of arterial CO_2 content ($PaCO_2$). Multidisciplinary research has implicated central biogenic amines in the regulation of cerebromicrocirculation. IS treatment resulted in increased escape latency and lowered levels of NE and 5-HT in the locus coeruleus but not in terminal fields in distant regions. This treatment also did not alter cerebral blood flow or capillary permeability in distant regions when compared with control rats. Thus, the discrete changes in NE and 5-HT in locus coeruleus induced by IS treatment is not reflected in changes in cerebral blood flow and the effective permeability of the blood-brain barrier.

Cerebral blood flow Animal model depression Learned helplessness Locus coeruleus Cerebrovascular permeability Monoamines

LEARNED helplessness has been proposed as an animal model of human affective illness. As such, it has been empirically useful and pharmacologically relevant due to its ability to discriminate drugs having antidepressant actions in man from other classes of psychotropic drugs. In this model, animals exposed to inescapable shock (IS) later show a deficit in escape responding. This behavior has been suggested to be mediated by central biogenic amine mechanisms. For example, the behavioral deficits observed in these animals have been related to modest reduction (i.e., approximately 20%) in norepinephrine in the locus coeruleus (LC), the major noradrenergic nucleus of the brain [19]. Moreover, tricyclic antidepressants (TCA) have been shown to reverse the escape-deficit in IS animals when given chronically, and this reversal has been related to TCA effects on central adrenergic systems (CAS; [12,18]). We proposed to test whether the NE reduction associated with behavior deficits leads to alteration in the functional integrity of the CAS.

To answer this question, the physiological functions the CAS subsumes in the brain must be known. Over the past decade, there has been a growing body of literature from several neuroscience disciplines that one function of the CAS is to modulate changes in the cerebral microcirculation in response to physiological changes such as metabolic demand [4, 13, 15, 16]. First, a close association has been observed between NE-positive fibers of central origin and cerebral capillaries, particularly in the paraventricular nucleus of the hypothalamus, using both light microscopic and electron microscopic techniques [4]. Second, LC neurons alter their firing rates in response to CO_2 administration which is a major determinant of cerebral blood flow (CBF) and the permeability-surface area product (PS) of the cerebral microcirculation [3,10]. Third, direct stimulation of LC neurons also produces alterations in both CBF and PS [16]. Fourth, somatic therapies for major affective disorders in man such as TCAs affect PS in a manner consistent with their known neurochemical effects on NE [15]. Fifth, abnormalities in cerebral microcirculatory response to CO_2 administration have been observed in another widely studied animal model of human affective disorder—the administration of tetrabenazine (TBZ) to rodents [14]. Of note, these TBZ-induced abnormalities occurred coincident with maximal depletion of the biogenic amines and was prevented by pretreatment with the TCA, amitriptyline.

The issue of whether microcirculatory abnormalities exist in the IS model takes on further significance given these

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findings in the TBZ model and reports of related abnormalities in patients with affective disorders [1, 2, 11, 17]. Given this body of literature, our objective was to test whether the reported behaviorally-induced reduction in NE levels in the LC resulted in abnormalities in cerebromicrocirculatory response to CO_2 administration and whether they were qualitatively similar to those observed in the TBZ model and affective disorders.

METHOD

Design and IS Induction

Subjects were male, Sprague-Dawley rats weighing 240–300 g. They were studied following three different treatments: (1) IS (n=12), (2) sham (n=5), and (3) untreated (n=15). Both IS and sham-treated groups were individually confined in a 50 cm(l) × 15 cm(w) × 50 cm(h) chamber with a 0.3 cm diameter grid (spaced 1.5 cm center to center) floor for sixty minutes [5]. The IS treatment consisted of alternating ten second periods of 0.8 mA scrambled, constant-current shock (Foringer scrambler and stimulator), and no shock. Sham-treated rats were confined in the same apparatus for an equivalent period but received no shock. Following treatment, the rats were removed and placed in a holding cage for one hour after which PS and CBF measurements, or behavioral measurements were made. Separate groups were used for the behavioral and the cerebrocirculation measurements to avoid any possible effects of behavioral testing. There was no sham-treated group for behavioral testing.

Determination of CBF and PS

This method has been published in detail elsewhere [7]. An abbreviated description follows: The rats were intubated, and passively ventilated with N_2O, O_2, and CO_2. CBF was physiologically altered by varying the amount of CO_2 in the inspired gas mixture. Body temperature was maintained at 37°C with a rectal thermistor and heat lamp. The femoral artery was cannulated to monitor arterial blood gases and mean arterial pressure (MAP). The femoral vein was cannulated to permit injection of the tracer solution (^{14}C-butanol, a freely-diffusible compound, and ^{3}H-water, a diffusion-limited compound).

Ten seconds after IV injection of the tracer bolus—sufficient time for the bolus to pass through the cerebral microcirculation—the animal was decapitated. The brain was rapidly removed and a coronal cut was made at the superior colliculus. The hindbrain and the forebrain sections were then divided into right and left halves by a midline sagittal cut. Both halves were then further divided by free-hand dissection into the following regions: cerebellum, medulla-pons, diencephalon, and rostral and caudal telencephalon. The samples from the left side were used for monoamine measurements while the right-side samples were used to measure PS and CBF. The latter samples were solubilized using Protosol (New England Nuclear Co.) and counted using standard dual-label liquid scintillation techniques.

The single-pass cerebral extraction of ^{3}H-water (Ew) in each region was determined by the following equation:

$$\text{(I)} \qquad E_w = \frac{(^{3}\text{H-water}/^{14}\text{C-butanol}) \text{ in brain}}{(^{3}\text{H-water}/^{14}\text{C-butanol}) \text{ in blood}}$$

CBF was determined by withdrawing a continuous femoral artery sample, from five seconds before tracer injection until decapitation:

$$\text{(II)} \quad \text{CBF} = \frac{^{14}\text{C-butanol in brain}}{^{14}\text{C-butanol in blood}} \times \text{sampling rate}$$

PS was calculated from Ew and CBF by the following equation:

$$\text{(III)} \qquad \text{PS} = -\ln(1\text{-Ew}) \times \text{CBF}$$

HPLC Analysis of Monoamines

The left side of the brains were dissected free-hand into the caudate/putamen, hypothalamus, thalamus, hippocampus, neocortex, and the locus coeruleus and kept in a −70°C freezer. At the time of assay, acetate buffer (400 μl, pH=5) and dihydroxybenzylamine (50 μl, 10 M) was mixed with the brain in a conical tube. After sonication, ascorbic acid oxidase (20 μl) was added to each conical and then centrifuged for 20 minutes at 20,000 rpm. Fifty μl of solution was injected onto a 25 cm ODS, C-18 reverse phase chromatographic column at a flow rate of 1 ml/min. The approximate elution time was 17 minutes at an average PSI of 2750 [8].

Behavioral Measurements

IS and untreated control rats were tested in a 100 cm(l) × 15 cm(w) × 50 cm(h) avoidance apparatus [6] with a 15 cm high barrier in the center. At the end of a 5 second warning signal (Sonalert), the rats were required to jump over the electrified barrier to the opposite side to escape the scrambled, electrified grid, then back, then to the opposite again (approximating a FR3 schedule). Latency to complete each trial was recorded with a maximum trial latency of 30 seconds. The intertrial interval was 60 seconds. Rats were given 20 trials each.

RESULTS AND DISCUSSION

Behavioral

Rats receiving IS induction had longer escape latencies (Mean±SEM=27.1±1.2) than did untreated control rats, 17.6±1.9; $t(6)=4.2$, $p<0.005$. This impaired escape performance due to IS induction replicates previous reports (e.g., [11]).

HPLC

Exposure to the IS treatment resulted in a reduction in NE and 5-HT in the locus coeruleus when compared to the same region in control rats, $t(5)=2.97$, $p<0.01$ and $t(6)=3.98$, $p<0.01$, respectively. No differences between IS treatment and control groups in levels of NE, 5-HT, or DA were found for the caudate, hypothalamus, thalamus, hippocampus and cortex brain regions using independent t-test analyses. These findings of reduced levels of NE and 5-HT in the locus coeruleus but not dopamine, as well as the finding of no depletions in the other brain regions, is consistent with reports by Weiss *et al.* [19,20]. And similar to Weiss [19], we did not find reduced levels of 5-HT in frontal cortex (cf., [18]).

CBF and PS

A multiple regression analysis was used to assess for significant changes in slope and/or intercept for CBF and PS. A single control group was formed from the sham and untreated groups since they did not differ statistically from each other on any of the measures. Comparisons were then made between the IS and pooled control group for each brain region.

In contrast to the impaired behavioral performance and

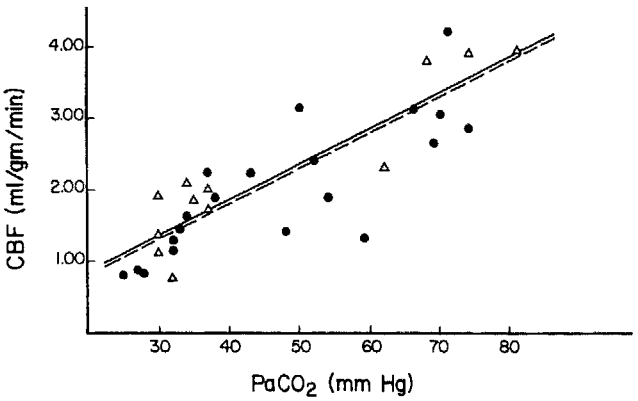

FIG. 1. Relation between CBF and $PaCO_2$ for both IS treatment (open) and controls (solid). There were no differences in slopes or intercepts for the two conditions in the total forebrain shown here or in other regions described in the text. Linear equations for IS and controls were similar, $y=-0.17+0.05X$ and $y=-.12+0.05X$, respectively.

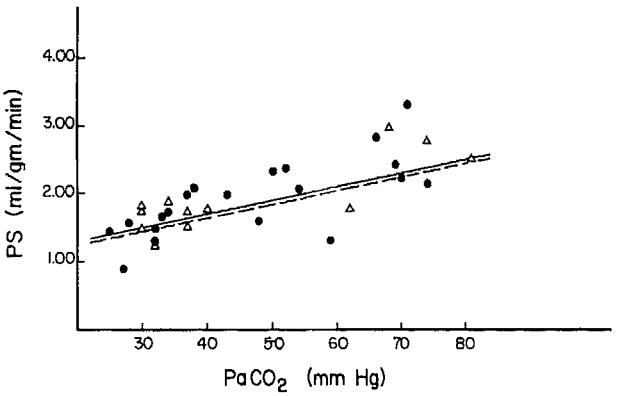

FIG. 2. Relation between PS and $PaCO_2$ for both IS treatment (open) and controls (solid). No differences in slopes or intercepts for the two conditions were found in the total forebrain or the other regions studied. Linear equations for IS and controls were also similar, $Y=0.85+0.02X$ and $y=0.9+0.02X$, respectively.

the reduced levels of 5-HT and NE in the locus coeruleus only, no differences between groups were found for the CBF and PS measures in any of the regions studied. For graphical data presentation, the results for the total forebrain (i.e., the pooled diencephalon and rostral and caudal telencephalon results) for each animal were plotted in Figs. 1 and 2.

Figure 1 represents the rate of change (i.e., the responsivity) of CBF as a function of $PaCO_2$ for the total forebrain region of control versus IS-treated rats. Analyses of each separate region as well as for the cerebellum and medulla pons regions were similar. Significant linear relationships were found for both IS and control groups, $R^2=.74$, $F(3,28)=26.9$, $p<0.001$. However, as can be seen from the scatterplot of CBF as a function of $PaCO_2$, multiple regression analyses indicated no difference in slope or intercept among any of the three treatment conditions.

A similar multiple regression analysis of PS for the various brain regions also revealed no intergroup difference (Fig. 2). Again, significant within group linear relationships were found, $R^2=.58$, $F(3,28)=12.76$, $p<0.001$.

In sum, we found that IS treatment produced (a) decrements in escape performance replicating previous behavioral work (e.g., [18–20]), (b) reduced levels of NE and 5-HT but not DA in the locus coeruleus, and (c) no change in the levels of any of the three biogenic amines in the terminal regions measured. We also extended this research to measures of cerebromicrovascular responsivity. We found that although the IS induction produces modest but reproducible changes in behavior and monoamines, a single-exposure to IS does not alter cerebral blood flow or the effective permeability of the blood-brain barrier (PS). It may be that chronic exposure to this IS paradigm is required to produce changes in CBF and PS. Conversely, physiological functions other than regulation of cerebromicrocirculation may be altered by acute IS induced reduction of NE and 5-HT in locus coeruleus. The point of this report is that there is the need to test whether an experimentally induced change in neurotransmitter levels, especially when the magnitude of the change is modest, has physiological consequences in terms of the functions subsumed by that chemically defined neural system. The failure to find abnormalities in cerebromicrocirculation in the IS model is somewhat of a surprise in light of preliminary reports [1, 2, 11, 17] of abnormalities in patients with affective illness.

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