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BRIEF COMMUNICATION

Portacaval Anastomosis in Rats: Effects on Behavior and Brain Serotonin Metabolism[1]

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MARTIN, J. R., J. DEDEK AND P. DRISCOLL. *Portacaval anastomosis in rats: Effects on behavior and brain serotonin metabolism.* PHARMACOL BIOCHEM BEHAV **18**(2) 269–272, 1983.—The behavior of male rats with chronic end-to-side portacaval shunts or control surgery were evaluated in a complex enclosed maze, an open-field arena, and a swim canal. Subsequently, serotonin and 5-hydroxyindoleacetic acid levels were measured for hypothalamus, striatum, hippocampus, and midbrain-medulla. Chronic portacaval anastomosis did not significantly alter any of the behavioral parameters evaluated in the diverse test situations studied, despite a significant elevation of 5-hydroxyindoleacetic acid levels in all brain regions analyzed and the elevation of serotonin levels in hypothalamus and midbrain-medulla.

Portacaval anastomosis Maze patrolling Open-field activity Swim endurance Serotonin
5-Hydroxyindoleacetic acid

CHRONIC liver dysfunction in man, especially with concomitant portosystemic shunting of the circulation, is frequently accompanied by hepatic encephalopathy. The rat with a surgically constructed portacaval anastomosis has served as a useful animal model for chronic liver insufficiency in man. Portacaval-shunted rats exhibit elevated plasma concentrations of aromatic amino acids and decreased concentrations of branched-chain amino acids [2] and increased brain concentrations of tryptophan, serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), glutamine, tyrosine and octopamine [5, 9, 18]. The pattern of biochemical alterations in patients exhibiting hepatic encephalopathy is generally similar to that observed in the portacaval-shunted rat [7,19].

Recently, attempts have been made to document the behavioral effects of portacaval shunting in rats and thereby determine the degree to which the pronounced cognitive and neuromuscular disturbances observed in patients with hepatic encephalopathy are duplicated in this animal model. Portacaval-shunted rats have been found to manifest abnormal patterns of general activity [3, 8, 15], attenuated startle response [17], and exaggerated consumption of various palatable solutions [11]. In addition, abnormal electroencephalographic patterns during both sleep and wakefulness have been reported as a consequence of experimental portacaval anastomosis in rats [6, 8, 12]. However, there is still considerable conflict among reports concerning the exact nature of the abnormalities, with different laboratories reporting alterations occurring in opposite directions.

The research conducted in our laboratories has failed to find any dramatic effects of portacaval anastomosis on general activity by rats in several diverse test situations [10]. Thus, it was considered desirable to do brain analysis to determine whether our experimental subjects show the well-documented alterations of brain 5-HT metabolism that have been linked to changes in ambulation in rats [15]. The rats in the present experiment were tested in several behavioral situations so as to demonstrate the absence of

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pronounced effects on diverse measures of activity despite the occurrence of abnormalities in brain neurochemistry in the same experimental subjects.

METHOD

Adult male Sprague-Dawley rats (Süddeutsche Versuchstierfarm, Tuttlingen, West Germany) received surgically created end-to-side portacaval shunts (N=8) or underwent sham surgery (N=8) according to a previously described procedure [11]. The subjects were individually housed in Macrolon plastic cages with sawdust bedding and given ad lib access to Nafag Laboratory Pellets (No. 890) and tap water during all testing. The ventilated quarters were maintained on a 12:12 hr light-dark cycle throughout the experiment. Behavioral testing was done 6–7 months postsurgery.

Three diverse test situations were chosen to evaluate the motor activity of the portacaval-shunted rats and their controls: (1) an illuminated open field, (2) a complex enclosed maze, and (3) a swim canal. The open-field and maze tests were done during the initial half of the dark phase and the swim endurance test was done during the middle part of the light phase of the light-dark cycle. Each rat received only a single session in each of the three test situations, with several days between successive test sessions. The rats first received a 95-min test in a brightly illuminated open field, as previously described [10]. The quadrant-crossing activity was evaluated during 7 separate 5-min periods, each followed by a 10-min interval during which measurements were not taken. The rats were next tested in an enclosed hexagonal maze which included an unilluminated central arena, as previously described [10]. The subject was placed into the maze through a door in the ceiling of the maze and the location of the rat was then automatically monitored during the 6-min test with a computer interfaced with 42 photocell units uniformly distributed throughout the maze alleys. The total activity was determined by totalling all photobeam interruptions and the explored area was provided by the total number of different photocell units (maximum 42) activated during the test session. The swim test was done in a 4-m long and 15 cm wide canal containing ambient temperature water, as previously described [1]. The subject was dropped into the water at one end and was required to swim to the other end of the canal to reach a platform and thereby escape the water. Each subject received 10 training trials on the day preceding the test session. Upon arrival at the platform, the rat was immediately returned to the other end of the canal and the next trial begun. The swim time for each test trial was recorded, as well as the total number of trials before exhaustion (inability to reach the platform within 1 min or the failure to continue swimming).

One day after the completion of the final behavioral test, the rats were killed by decapitation. The brain was rapidly removed and the hypothalamus, striatum, hippocampus, and midbrain-medulla (including pons) were dissected out on an iced platform and then frozen at −20°C. Analysis was subsequently done using the spectrofluorometric method of Curzon and Green [4] with 5-HT and 5-HIAA being isolated on small Sephadex G-10 columns as described elsewhere [13]. The presence of a functional shunt in each of the rats of the portacaval-shunted group and the absence of any obvious vascular abnormalities in the control group was verified in a necropsy. The behavioral and neurochemical data for the portacaval-shunted and sham-operated groups were compared using the Mann-Whitney U test. Statistical tests for the behavioral data were based upon two-tailed p-values and those for the neurochemical data were based upon one-tailed p-values. Correlational analysis was done with the Spearman Rank Correlation test and subsequent evaluation of the statistical significance of these correlations was done according to Siegel [14].

RESULTS

Table 1 shows the concentrations of 5-HT and 5-HIAA in the hypothalamus, striatum, hippocampus, and midbrain-medulla. The level of 5-HIAA was significantly elevated in rats with portacaval shunts relative to their sham-operated controls for all four brain regions and that of 5-HT was significantly increased in the portacaval-shunted rats in comparison to controls only for hypothalamus and midbrain-medulla. Although the increase in 5-HT concentration in the various brain regions was small relative to the increases in 5-HIAA concentration, these results are consistent with enhanced 5-HT turnover.

There was no significant effect of portacaval anastomosis on total open-field quadrant crossings, open-field quadrant crossings following the initial 15 min in the apparatus (the index of locomotion used in [15]), total maze activity, explored maze area, and number of swim trials to exhaustion. The total duration of swimming to exhaustion (mean±SD) was greater for sham-operated rats (31.4±7.5 min) than for rats with portacaval shunts (19.2±4.5 min), but this difference was not statistically significant ($p<0.06$). It should be noted that rats with portacaval shunts weighed 30% less than their controls and, thus, an index of work (body weight × minimum distance swum) was also calculated. However, there was no significant difference between portacaval-shunted rats and their controls for this index; the rats with portacaval shunts weighed less but completed nonsignificantly more swim trials (each trial involved swimming at least 4 m) than their controls.

The correlation between regional 5-HT and 5-HIAA levels and the two measures of open-field activity were calculated separately for the portacaval-shunted group and for the control group. None of these correlations reached statistical significance, and the pattern of correlations did not parallel that previously reported [15].

DISCUSSION

Consistent with earlier research [2, 5, 15, 16], the concentration of 5-HIAA in rats with portacaval shunts was significantly elevated over control levels for hypothalamus, striatum, hippocampus, and midbrain-medulla. Serotonin concentration was significantly increased in portacaval-shunted rats relative to controls only in the hypothalamus and midbrain-medulla. The rats with experimental portacaval shunts used in the present behavioral tests, thus, exhibited well documented alterations in 5-HT and 5-HIAA levels in the brain. In the present investigation, behavior and neurochemical concentrations were not significantly correlated. However, Tricklebank *et al.* [15] have noted that ambulation was mostly clearly correlated with tryptophan concentration, which was not measured in the present study.

In contrast to some reports [15,16], no pronounced differences in general activity were observed between control rats and rats with portacaval shunts in the open-field test used in the present experiment, as well as in an earlier study [10]. Even the analysis of ambulation data beginning only after the

TABLE 1

REGIONAL BRAIN CONCENTRATIONS OF SEROTONIN (5-HT) AND 5-HYDROXYINDOLEACETIC ACID (5-HIAA) IN PORTACAVAL-SHUNTED AND CONTROL RATS

	Control	Porta-caval	Percent Increase	*p* Value
5-HT (ng/g wet wt)				
Hypothalamus	781 ± 39	899 ± 29	15	<0.04
Striatum	458 ± 23	474 ± 31	3	NS
Hippocampus	398 ± 30	415 ± 37	4	NS
Midbrain-medulla	476 ± 38	610 ± 20	28	<0.03
5-HIAA (ng/g wet wt)				
Hypothalamus	455 ± 32	1144 ± 66	151	<0.001
Striatum	446 ± 25	784 ± 46	76	<0.001
Hippocampus	235 ± 18	440 ± 36	88	<0.001
Midbrain-medulla	284 ± 14	962 ± 57	239	<0.001

The concentration values are given as mean ± SEM.

initial 15 min of exposure to the open field (similar to the analysis in [15,16]) did not result in any significant difference between experimental and control groups. It does not appear likely that the longer postsurgery interval in the present study greatly influenced the level of activity because the absence of effects of portacaval anastomosis on open-field and maze behavior have been observed in this laboratory following 1–2 months [10] and 6–7 months postsurgery intervals (present study). Furthermore, portacaval anastomosis did not significantly alter swim endurance in the present investigation. However, in this and in many other studies, experimental portacaval anastomosis produced chronic body weight reduction that may contribute to the biochemical alterations produced and possibly to the behavioral alterations observed in some test situations.

Despite the presence of pronounced alterations in brain levels of 5-HT and 5-HIAA, no dramatic changes in activity in several diverse test situations were observed in the present series of experiments. Data from this laboratory do not indicate that the behavior of rats with portacaval shunts is completely normal since such subjects have been found to be hypoactive in relatively long test sessions in a hexagonal alleyway [10]. However, the pronounced abnormalities in brain serotonin metabolism that follow the construction of a portacaval shunt sometimes accompany, but do not necessarily produce any dramatic alterations in the general activity of rats.

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