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Decreased Startle Reactivity in the End-To-Side Portacaval Shunted Rat[1]

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WARBRITTON, J. D., III, M. A. GEYER, B. JEPPSSON AND J. E. FISCHER. *Decreased startle reactivity in the end-to-side portacaval shunted rat.* PHARMAC. BIOCHEM. BEHAV. 12(5) 739–742, 1980.—A rat with an end-to-side portacaval anastomosis (PCA) is an extensively used experimental animal for characterization of biochemical alterations following diversion of portal blood flow from the liver; but few behavioral abnormalities have been detected. Biochemical changes observed in rats after PCA include increased plasma and brain levels of the serotonin precursor tryptophan, with increased brain serotonin and its metabolite 5-hydroxyindoleacetic acid. Accumulating evidence indicates that serotonin may be involved in the modulation of the startle response in the rat. In this study, the magnitude of startle responses to both tactile and auditory stimuli were shown to be abnormally decreased in chronic PCA rats. The simplicity of this animal model and the reproducibility of the effect suggest that evaluation of startle responses in rats with PCA may enable assessments of the efficacy of putative therapeutic strategies for the treatment of some of the dysfunctions associated with hepatic encephalopathy.

Portacaval shunt Hepatic encephalopathy Serotonin Tryptophan Startle response Habituation Sensitization

IN man, cirrhosis of the liver is frequently accompanied by symptoms of neurological dysfunction, including affective changes, dementia, disorientation, somnolence and lethargy. This syndrome, hepatic encephalopathy, may progress to stupor, coma, and ultimately death. The rat with a surgically created portacaval anastomosis has been used extensively as an animal model of chronic liver insufficiency [8].

The neurochemical changes observed in rats following portacaval anastomosis (PCA) tend to parallel changes seen in humans with cirrhosis of the liver. Both exhibit gross hyperammonemia [4,20] and altered plasma amino acid profiles, with increased levels of aromatic amino acids (tryptophan, tyrosine and phenylalanine) and decreased concentrations of branched-chain neutral amino acids (leucine, isoleucine and valine) [7, 15, 22, 25].

Potential relationships between biochemical alterations and behavioral disturbances observed in patients with chronic liver disease are difficult to evaluate in clinical studies. However, the rat with PCA is amenable to detailed behavioral study. Indeed, a variety of behavioral alterations have been reported, including disrupted sleep patterns and circadian rhythms, decreased responses to electric shock and decreased locomotor activity [1, 2, 3, 21, 26, 29].

It has been suggested that some of the behavioral changes observed in rats with PCA may reflect, in part, increased brain levels of serotonin (5-HT) [1,26]. The rat startle response to novel stimuli seems to be a sensitive behavioral index of alterations in central serotonergic neurotransmission, as increased brain 5-HT is associated with decreased responsivity [10] and manipulations which decrease 5-HT levels or cause a functional antagonism of brain 5-HT tend to increase startle responses [6]. In the present study, the effects of PCA on the rat startle response to both tactile and auditory stimuli have been tested.

METHOD

Animals

The animals were 34 experimentally naive male Sprague-Dawley rats (250–300 g) from Charles River Breed-

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ing Laboratories. They were randomly divided into 2 groups for either sham or PCA operations (see below). One week after surgery in Cincinnati, OH, the animals were flown to San Diego, CA, where they were housed 2 to a cage on a 12/12 hr light/dark cycle for 2 weeks prior to behavioral testing. Water and Purina Rat Chow were continuously available.

Surgery

The rats were anesthetized with ether and a portacaval anastomosis (PCA) was created by the method previously described [9]. Using clean, but not sterile technique, the abdominal wall was opened with a midline vertical incision; the portal vein was exposed, dissected and ligated. The clamped portal vein was then cut free and fed through a small piece of teflon tubing which had previously been sculpted to form a "button" of the type used for vascular anastomoses. The vein was everted over the button and secured with a silk ligature; the vein-button assembly was then anastomosed to the partially clamped vena cava with a purse string suture. A sham operation consisted of the same preparation with clamping of the portal vein and vena cava but without anastomosis.

Apparatus

Startle response magnitudes were measured in stabilimeters consisting of 4 in. diameter Plexiglas cylinders held at top and bottom within a rigid frame by large rubber stoppers. Displacements of the cylinder by the animal were detected by a ceramic phonograph cartridge mounted on the outer frame as described previously [12]. Each msec for 250 msec following the onset of the stimulus, the signal from the cartridge was sampled by an analog-to-digital converter and stored on cassette tape by an Intel 8080 computer system. The magnitude of each response was defined as the difference between the minimum and maximum readings during this 250 msec interval.

The computer also controlled the presentations of either tactile or auditory stimuli. The tactile stimuli consisted of 50 msec puffs of air at a nominal pressure of 37.5 lbs/in^2 delivered simultaneously to two chambers through a solenoid with an 1/8 in. orifice. The tubes were positioned so that the air-puffs typically hit the middle of the animal's back [12]. The auditory stimuli consisted of 50 msec tones (1000 Hz tone at 115 dBA) with 0.5 msec rise-time against a continous background noise level of 70 dBA. Sound levels were measured with a Quest Sound Level Meter.

Behavioral Procedure

All 34 animals were first tested with tactile stimuli 28 days after surgery. Five minutes after placement in the stabilimeter, 81 air-puffs were presented at 15 sec intervals. One week later 30 animals were tested similarly for 49 trials with acoustic stimuli.

Data Analysis

The data were condensed into blocks of 10 (tactile) or 6 (acoustic) trials each, with the initial response being considered separately. Habituation was assessed by a repeated measures analysis of variance (ANOVA) over trial blocks and by the difference between the first and last trial blocks. A measure of sensitization was defined as the difference between the first trial and the first trial block [11]. Overall reactivity was assessed by a one-way ANOVA on the grand mean across all trial blocks for each subject. Pearson's statistic was used for correlational analyses [30].

RESULTS

The results of the tactile startle test are shown in Fig. 1 as the first response and the mean of each block of 10 trials. A mixed-design ANOVA revealed significant effects of the treatment, $F(1,32)=6.03$, $p<0.05$, and of trial blocks, $F(7,224)=12.62$, $p<0.001$, with no significant treatment-by-trials interaction, $F(7,224)=0.3$, n.s.. Similarly, the average startle magnitude across all trials was significantly lower in the PCA-operated rats, $F(1,32)=6.33$, $p<0.05$. No significant differences were found on the first response, or on the sensitization and habituation scores.

The results of the acoustic startle test are shown in Fig. 2. The mixed design ANOVA again demonstrated a significant treatment effect, $F(1,28)=5.75$, $p<0.05$, a significant trials effect, $F(7,196)=11.04$, $p<0.001$, and no interaction, $F(7,196)=1.6$, n.s. As with tactile startle, the overall means were significantly lower in the PCA-operated animals, $F(1,28)=5.49$, $p<0.05$, and no significant differences were found on the first response or on the sensitization and habituation scores.

To assess the degree of correspondence between the 2 behavioral tests, the overall means for each animal on both tests were used in a correlation analysis. Across both groups, tactile and auditory startle were significantly correlated ($r=0.445$, $N=30$, $p<0.02$).

DISCUSSION

The results demonstrate that rats with PCA exhibit abnormally decreased startle responses compared to controls, evaluated by either tactile or auditory stimulus modalities. Startle responses to tactile stimuli significantly predict responses to auditory stimuli for an individual animal. Both PCA and Sham-operated rats habituated comparably with either stimulus modality.

Neurochemical studies in rats have demonstrated elevated brain concentrations of the aromatic amino acids, tryptophan, tyrosine, and phenylalanine, following PCA [24]. This elevation appears to involve an increase in the activity of the neutral amino acid blood-brain-barrier transport system, thereby increasing the availability of the neutral amino acids to the concentrative, active transport systems of the brain cells [16]. In contrast to the biosynthesis of catecholamines, the synthesis of indoleamines, particularly the rate-limiting enzyme in serotonin biosynthesis, tryptophan hydroxylase, is normally limited by substrate availability *in vivo* [19]. Hence, the higher brain tryptophan levels produce increases in 5-HT particularly in the medulla, pons and midbrain, regions rich in serotonergic cell bodies. Furthermore, 5-hydroxyindoleacetic acid (5-HIAA) is elevated throughout the brain, suggesting an increased 5-HT turnover [5].

Clinical studies have revealed that patients with chronic liver disease exhibit elevated CSF levels of tryptophan, 5-HIAA, tyrosine and homovanillic acid, the precursors and metabolites of 5-HT and dopamine, respectively [18, 23, 31, 32]. A recent human post-mortem study suggests that disturbances in central 5-HT metabolism may be partly responsible for encephalopathic symptoms. Increased brain levels of 5-HIAA and tryptophan were found in patients who died in hepatic coma; but in encephalopathic patients treated with

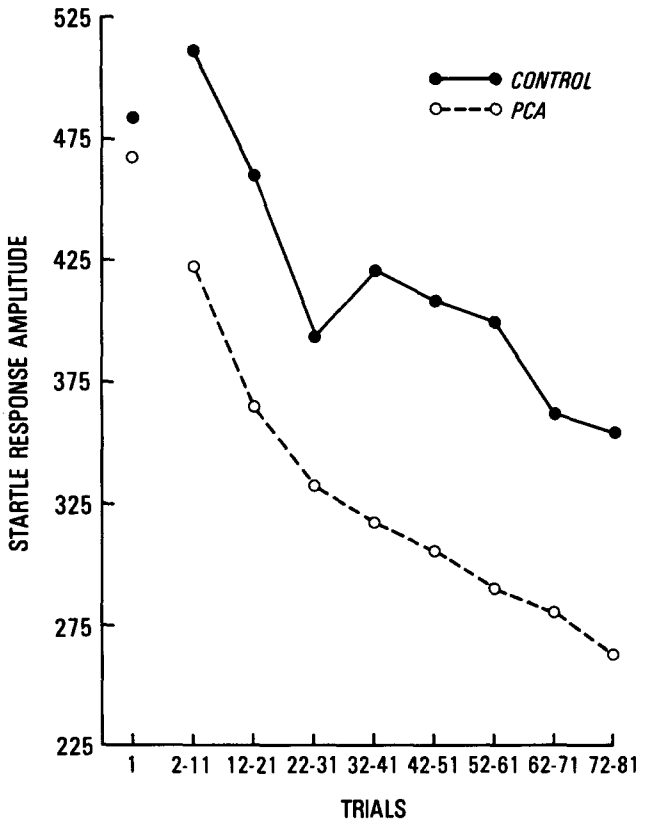

FIG. 1. Mean startle response magnitudes on Trial 1 and subsequent blocks of ten trials each for sham- and PCA-operated rats using tactile stimuli. The startle magnitudes across all trials were significantly lower ($p<0.05$) in the PCA-operated rats. N=17 for each group.

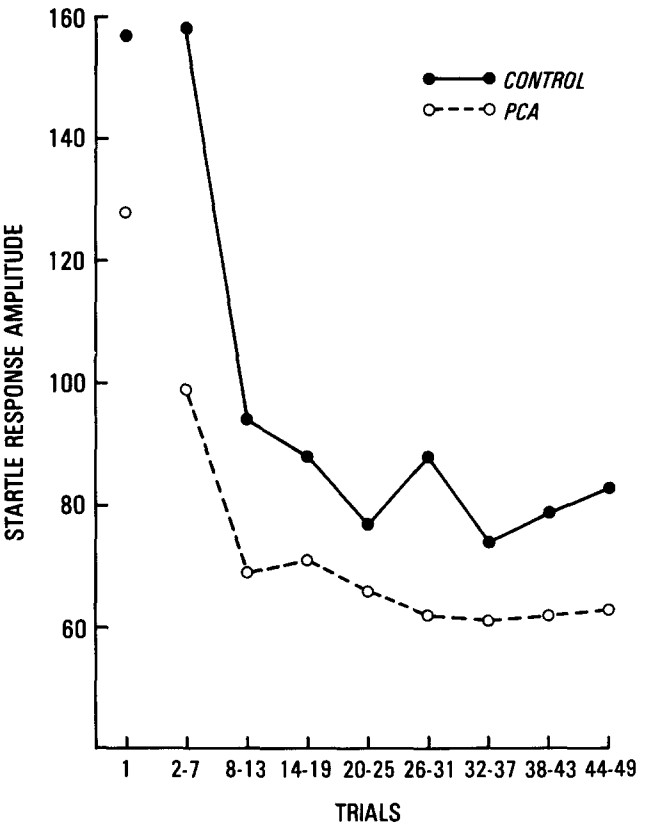

FIG. 2. Mean startle response magnitudes on Trial 1 and subsequent blocks of six trials each for sham- and PCA-operated rats using auditory stimuli. The startle magnitudes across all trials were significantly lower ($p<0.05$) in the PCA-operated rats. N=15 for each group.

parenteral branched-chain neutral amino acids who became non-comatose but later died from gastrointestinal hemorrhage, these changes were not apparent [17]. In tryptophan loading studies with normal volunteers, sedation, lethargy, alterations in mood and behavior, and electroencephalographic changes have been observed as the levels of plasma free tryptophan increase [13,28]. Similar phenomena are noted in patients with hepatic encephalopathy [14].

The rat with PCA does not exhibit signs comparable to human portalsystemic encephalopathy but behavioral abnormalities consistent with altered neurotransmitter metabolism have been reported. Electroencephalographic studies have revealed a decrease in the total duration of slow wave and REM sleep stages in rats with PCA [21]. Experiments using electrical stimulation of the brainstem reticular formation during slow wave sleep have found that the stimulus intensity necessary to provoke cortical awakening is significantly decreased after PCA [1]. A recent study utilizing 24-hr automated monitoring of spontaneous motor activity has revealed disrupted circadian rhythms in rats with PCA [3], consistent with the electrical findings of disturbed sleep patterns. Other behavioral changes observed in rats with PCA include decreased responses to electric shock [26,29], decreased ambulation in both home cage and open field settings [2,29] and increased frequency of social activity [29].

The findings of decreased tactile and auditory startle responsivity presented here are consistent with the suggested influence of central serotonergic transmission on the rat startle response, as substantial evidence indicates that treatments which increase brain 5-HT levels, including PCA, reduce responses to tactile [10] and auditory [6] stimuli. Available evidence suggests that brain 5-HT may function as a *modulator* of the general level of reactivity to tactile stimuli, without affecting the presumably more complex processes of habituation and stimulus sensitization [11]. Previous studies with acoustic startle indicated that serotonergic manipulations affect sensitization to the background noise [6], but this type of sensitization was not assessed in the present study.

Rats with PCA do not exhibit gross signs of central nervous system dysfunction and few behavioral abnormalities are apparent by casual observation. However, with testing, decreased responsivity to various sensory stimuli appears to be a consistent and reliable finding. Since alterations in startle responding are produced by abnormalities in neurotransmitter metabolism common to both rats with PCA and humans with chronic liver disease, startle measures in rats with PCA may provide a useful animal model of hepatic dysfunction in man. The simplicity of this animal model and the reproducibility of the effect suggest that evaluation of startle responses in rats with PCA may enable assessments of the efficacy of putative therapeutic strategies for the treatment of some aspects of hepatic encephalopathy.

REFERENCES

1. Beaubernard, C., F. Salomon, D. Grange, M. J. Thangapregassam and J. Bismuth. Experimental hepatic encephalopathy. Changes of the level of wakefulness in the rat with portacaval shunt. *Biomedicine* **27:** 169–171, 1977.
2. Bloxam, D. L., G. Curzon, B. D. Kantamaneri and M. D. Tricklebank. Effects of tryptophan and portacaval anastomosis on activity and brain tryptophan metabolism. *Br. J. Pharmac.* **60:** 277, 1977.
3. Campbell, A., V. Ziparo, J. H. James and J. E. Fischer. Spontaneous motor activity after portacaval anastomosis in rats. *Eur. J. Pharmac.*, submitted.
4. Cremer, J. E., D. F. Heath, H. M. Teal, M. S. Woods and J. B. Cavanaugh. Some dynamic aspects of brain metabolism in rats given a portacaval anastomosis. *Neuropath. appl. Neurobiol.* **1:** 293–311, 1975.
5. Cummings, M. G., P. B. Soeters, J. H. James, J. M. Keane and J. E. Fischer. Regional brain indoleamine metabolism following chronic portacaval anastomosis in the rat. *J. Neurochem.* **27:** 501–509, 1976.
6. Davis, M. Neurochemical modulation of the startle reflex: Review and theory. *Neurosci. Biobehav. Rev.*, in press.
7. Fischer, J. E., N. Yoshimura, A. Aguirre, J. H. James, M. G. Cummings, R. M. Abel and F. Deindoerfer. Plasma amino acids in patients with hepatic encephalopathy: Effects of amino acid infusion. *Am. J Surg.* **127:** 40–47, 1974.
8. Fischer, J. E. *Animal Models in Psychiatry and Neurology.* New York: Pergamon Press, 1977, pp. 385–390.
9. Funovics, J. M., M. G. Cummings, L. Shuman, J. H. James and J. E. Fischer. An improved nonsuture method for portocaval anastomosis in the rat. *Surgery* **77:** 661–664, 1975.
10. Geyer, M. A., J. D. Warbritton, D. B. Menkes, J. A. Zook and A. J. Mandell. Opposite effects of intraventricular serotonin and bufotenin on rat startle responses. *Pharmac. Biochem. Behav.* **3:** 687–691, 1975.
11. Geyer, M. A., A. Puerto, D. B. Menkes, D. S. Segal and A. J. Mandell. Behavioral studies following lesions of the mesolimbic and mesostriatal serotonergic pathways. *Brain Res.* **106:** 257–270, 1976.
12. Geyer, M. A., L. R. Petersen, G. J. Rose, D. D. Horwitt, R. K. Light, L. M. Adams, J. A. Zook, R. L. Hawkins and A. J. Mandell. The effects of lysergic acid diethylamide and mescaline-derived hallucinogens on sensory-integrative function: Tactile startle. *J. Pharmac. exp. Ther.* **207:** 837–847, 1978.
13. Greenwood, M. H., J. Friedel, A. J. Bond, G. Curzon and M. H. Lader. The acute effects of intravenous infusion of l-tryptophan in normal subjects. *Clin. Pharmac. Ther.* **16:** 445–464, 1974.
14. Hoyumpa, A. M., P. V. Desmond, G. R. Avant, R. K. Roberts and S. Schenker. Hepatic encephalopathy. *Gastroenterology* **76:** 184–195, 1979.
15. James, J. A., J. M. Hodgman, J. M. Funovics, N. Yoshimura and J. E. Fischer. Brain tryptophan, plasma free tryptophan and distribution of plasma neural amino acids. *Metabolism* **25:** 471–476, 1976.
16. James, J. H., J. Escourrou and J. E. Fischer. Blood-brain neutral amino acid transport activity is increased after portacaval anastomosis. *Science* **200:** 1395–1397, 1978.
17. Jellinger, K., P. Riederer, G. Kleinberger, St. Wuketich and P. Kothbauer. Brain monoamines in human hepatic encephalopathy. *Acta neuropath.* **43:** 63–68, 1978.
18. Knell, A. J., A. R. Davidson, R. Williams, B. D. Kantamaneni and G. Curson. Dopamine and serotonin metabolism in hepatic encephalopathy. *Br. Med. J.* **1:** 549–551, 1974.
19. Lovenberg, W. and S. J. Victor. Regulation of tryptophan and tyrosine hydroxylase. *Life Sci.* **14:** 2337–2353, 1974.
20. McDermott, W. V. The role of ammonia intoxication in hepatic coma. *Bull. N.Y. Acad. Med.* **34:** 352–365, 1958.
21. Monmaur, P. C., C. Beaubernard, F. Salomon, D. Grange, M. J. Thangapregassam and H. Bismuth. Encephalopathie hepatique experimentale. I—modifications de la durée des différents états de sommeil diurne chez le rat aveq anastomose porto-cave. *Biol. Gastroenterol.* **9:** 99–103, 1976.
22. Ono, J., D. G. Hutson, R. S. Dombro, J. U. Levi, A. Livingstone and R. Zeppa. Tryptophan and hepatic coma. *Gastroenterology* **74:** 196–200, 1978.
23. Record, C. O., B. Buxton, R. A. Chase, G. Curzon, I. M. Murray-Lyon and R. Williams. Plasma and brain amino acids in fulminant hepatic failure and their relationship to hepatic encephalopathy. *Eur. J. clin. Invest.* **6:** 387–394, 1976.
24. Rosen, H. M., P. B. Soeters, J. J. James, J. Hodgman and J. E. Fischer. Influences of exogenous intake and nitrogen balance on plasma and brain aromatic amino acid concentrations. *Metabolism* **27:** 393–404, 1978.
25. Salerno, F., F. S. Dioguardi and R. Abbiati. Tryptophan and hepatic coma (letter). *Gastroenterology* **75:** 769–770, 1978.
26. Salomon, F., C. Beaubernard, M. J. Thangapregassam, D. Grange and H. Bismuth. Encephalopathie hepatique experimentale. II—Etude de la réaction a'la douleur chez le rat avec anastomose porto-cave. *Biol. Gastroenterol.* **9:** 105–108, 1976.
27. Simert, G., A. Nobin, E. Rosengren and J. Vang. Neurotransmitter changes in the rat brain after portacaval asastomosis. *Eur. Surg. Res.* **10:** 73–75, 1978.
28. Smith, B. and D. J. Prockop. Central-nervous-system effects of ingestion of l-tryptophan by normal subjects. *New Engl. J. Med.* **267:** 1338–1341, 1962.
29. Tricklebank, M. D., J. L. Smart, D. L. Bloxam and G. Curzon. Effects of chronic experimental liver dysfunction and l-tryptophan on behavior in the rat. *Pharmac. Biochem. Behav.* **9:** 181–189, 1978.
30. Winer, B. *Statistical Principles in Experimental Design*, 2nd edition. New York: McGraw-Hill Book Company, 1971.
31. Young, S. N., E. Garelis, S. Lal, J. B. Martin, P. Molina-Negro, R. Ethier and T. L. Sourkes. Tryptophan and 5-hydroxyindoleacetic acid in human cerebrospinal fluid. *J. Neurochem.* **22:** 777–779, 1974.
32. Young, S. N., S. Lal, T. L. Sourkes, F. Feldmuller, A. Aronoff and J. B. Martin. Relationships between tryptophan in serum and CSF and 5-hydroxyindoleacetic acid in CSF of man: Effect of cirrhosis of liver and probenicid administration. *J. Neurol. Neurosurg. Psychiat.* **38:** 322–330, 1975.