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Portacaval Shunt in the Rat: Selective Alterations in Behavior and Brain Serotonin

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BENGTSSON, F., A. NOBIN, B. FALCK, F. H. GAGE AND B. JEPPSSON. *Portacaval shunt in the rat: Selective alterations in behavior and brain serotonin.* PHARMACOL BIOCHEM BEHAV **24**(6) 1611–1616, 1986.—Portacaval shunted (PCS) rats and sham-operated controls were investigated for spontaneous activity, exploration, somatosensory reactivity, swim latencies in a water maze, motor coordination, and passive avoidance 2 to 3 weeks after operation. The rats were subsequently decapitated and indole metabolism was investigated in different brain regions. The results showed that shunted rats were impaired in both open field tests (spontaneous activity and exploration) and in somatosensory reactivity (latency to respond, maximal response and integrated response). Results from motor coordination tasks and learning and memory tests (water maze and passive avoidance) did not demonstrate differences between the groups. There was an increased brain indolamine metabolism in PCS compared to sham-operated rats. No correlation between the behavioral impairment and the altered indolamine metabolism could be demonstrated with multiple correlation analysis.

Portal-systemic encephalopathy Portacaval shunt Open field behavior Startle response
Motor coordination Learning and memory Brain serotonin metabolism

PORTAL-SYSTEMIC encephalopathy (PSE) occurs in patients with liver failure or portal-systemic shunting of blood. The syndrome of PSE includes symptoms of mental and neuro-muscular dysfunctions. PSE is, in its early stages, fully reversible, but can progress to stupor, coma, and ultimately death.

The behavioral disturbances in PSE are present concomitant with many cerebral metabolic disorders and much effort has been made to elucidate any pathogenetic interrelation of these alterations [12]. No clear basis for the pathogenesis and underlying pathophysiological mechanisms of PSE has been presented so far. In addition, many of the previous reports on different changes in behavior and metabolism in patients or experimental animals have provided conflicting data [2, 6, 14, 18, 22, 27].

The commonly used model for experimental studies of PSE is a chronic end-to-side portacaval shunt (PCS) in rats. These rats exhibit a variety of biochemical changes strikingly similar to those found in humans with chronic hepatic disease and PSE [8]. Present available data show decreased open field activity in PCS rats and our previous results have supported this behavioral deficiency in the experimental PSE model. This decreased locomotor activity is present simultaneously with an increased brain indolamine metabolism in the PCS rats [2]. The basis for this change in indole metabolism has been suggested to be the increased level of brain aromatic amino acids such as tryptophan, the precursor for the serotonergic neurotransmitters [4,15]. A causal relation between the disturbed behavior and increased brain indolamine metabolism, has, however, not been clearly demonstrated [21,25].

The objective of the present experiment was to provide a more complete examination of the behavioral deficits in this animal model of PSE, and to determine whether the changes in behavior are directly correlated with indolamine turnover in the brain of these impaired rats.

METHOD

Subjects

A total of 18 female Sprague-Dawley rats, at time of operation weighing 180–200 g, were used in this experiment. The rats were housed in standard laboratory cages in groups of 4 animals, with ad lib access to food and water, and maintained on a 12:12 light:dark cycle. In the beginning of the experiment, 12 rats were operated with PCS and 6 rats underwent sham operations (sham) (see surgery below). They were kept for a period of 3 weeks before sacrifice and quantitation of brain indoles. The behavioral tests were performed during 7 days immediately preceding the killing. The tests were undertaken in the following order: Motor coordination, water maze, step-through passive avoidance, spontaneous activity and exploration (tested in the dark cycle) and somatosensory reactivity.

Surgery

A chronic end-to-side portacaval anastomosis was created under ether anaesthesia using a clean but not-

sterile technique [16]. The abdomen was opened by a midline incision and the portal vein was ligated distally, divided and anastomosed to the inferior caval vein just cranial to the renal veins. The anastomosis was performed with a continuous 9-0 silk. The patency of the anastomosis was evident from the rapid return of congested blood from the intestines. The sham-operation included handling of the viscera, dissection and clamping of the portal and caval veins but without the anastomosis. The effectiveness of the portacaval shunt was verified at sacrifice in the operation microscope (12× magnification). All shunts were judged to be patent morphologically in this way. Ammonia levels in plasma also served as a check on shunt patency (see below).

Behavioral Tests

Spontaneous activity and exploration in an open field. Rats were tested in 8 automated open field boxes (50×50×50 cm) made of grey plastic. In each box, 4 evenly spaced photo cell beams perpendicular to each wall and 2 cm above the floor divided the box into 25 10×10 cm squares. Each photo cell beam interruption was automatically registered as an activity count by on-line connection to an ABC 80 microprocessor. The floor was raised 5 cm from the base of the box and contained 16 evenly spaced 2 cm diameter holes in a 4×4 array. An additional row of 4 photo cell beams lay 1 cm below the holes and automatically recorded each exploratory nose-poke. Each animal was tested in the dark for 1 hour.

Somatosensory reactivity. A startle box apparatus was used similar to one described by Turner and Gage [23]. This permits measurements of the unconditioned startle response to somatosensory stimulation. The apparatus allows for the measurement of (1) the latency to respond to stimulus (with threshold established at a preset level), (2) the integral of the response over the time the initial response was above threshold and (3) the maximal amplitude of the response, which is the peak height of the integrated response. The startle was induced by a 0.5 mA, 100 msec unscrambled electrical pulse through the brass rods of the grid floor of the box. The mean response on each measure over 5 successful trials was taken as the reaction of the animals. Previous experience with this stimulus level has demonstrated that the magnitude of the response allows for increases and decreases in somatosensory reaction and that there is no apparent habituation to the response over 12 presentations.

Water maze. The water maze test was adapted from a description by Morris [19]. The test apparatus comprised a large circular pool of polyvinyl chloride (140 cm dia. × 45 cm high) filled with water to a depth of 30 cm. The water was made opaque by the addition of approximately 1/2 liter dried milk powder, and was thermostatically maintained at 26±1°C. An 11×11 cm transparent platform, 29 cm high, was placed in a fixed location in the tank, 1 cm below the water surface. The platform was not visible from just above water level (at least to the experimenter's eye), and additional transfer trials indicated that escape onto the platform was not achieved by visual or other proximal cues. Many extra-maze cues surrounded the maze and were available for the rats to use in locating the escape platform.

Each trial involved placing the rats into the water close to and facing the wall of the pool in one of 4 equally spaced locations. The rats were allowed to swim freely around the pool until they found the platform, onto which they would promptly climb to escape from the water. If a rat failed to locate the platform within 120 sec, it was placed there by the experimenter. The intertrial interval was 60 sec, during which the rat remained on the platform. Each rat received 8 trials per day for 5 days. On the first 36 trials the platform remained in a constant location equidistant from the center and the edge of the pool, in the center of one quadrant. For the final 4 trials the platform was removed, which enabled a determination of whether the animals had learned the task using spatial or proximal cues.

On each trial the latency to escape onto the platform was measured by the stopwatch. The mean value was calculated for each rat after a block of 4 consecutive trials. On trials 37–40 the platform was removed and the number of times the rats passed over the location where the platform had been was counted and used as an index of spatial activity.

Motor coordination. Three measures of motor coordination were adopted, based on a previously described test [24]. (1) A square bridge: a 60 cm long wooden bridge of 2×2 cm cross section was suspended between safety platforms 60 cm above the bench surface. The rat was placed in the middle of the bridge, and the latency to reach the safety platform or to fall was recorded. (2) A round bridge: this test was identical to the first except that the bridge was made of wood dowel rod of 2 cm diameter circular cross section. (3) Wire Suspension: taut, plastic-coated wire was stretched horizontally 60 cm above the bench surface. Rats will clasp this wire with their forepaws and hang suspended or try to climb up. The latency to fall was recorded.

A foam rubber pad was placed beneath the bridge or wire to cushion the rats' falls. On each test a maximum latency of 120 sec was allowed for the animal to fall. Any rat which took longer, or reached one of the safety platforms of the bridges, was attributed the maximum score (120 sec). Each rat was tested on each of the tests 3 times in cyclical order.

Step-through passive avoidance. The step-through passive avoidance chamber made of grey plastic was divided into 2 compartments of dimensions 30×30×30 cm by a guillotine door. The start box was brightly lit, whereas the shock box had a roof that made it dark and only dimly lit through the door of the start box. Shock (AC) was delivered to a grid floor of the shock compartment by means of a shock generator/scrambler. On the training day the rat was placed in the start compartment, and 30 sec later the guillotine door was raised. Latency to enter the dark compartment was recorded. When the rat stepped through, defined as all 4 paws beyond the threshold of the door, the door was lowered and the rat received 3 consecutive 1 sec foot shocks at an intensity reading of 1 mA on the shocker meter. The rat remained in the shock box for 30 sec and was then returned to its ordinary cage. Twenty-four hours later the rat was once again placed in the start box, and after a 30 sec interval, the guillotine door was raised. Latency to enter the dark compartment was recorded. If the rat did not step through in 120 sec, the test was terminated.

Indole Quantitations in Different Brain Regions

5-Hydroxytryptophan (5-HTP), 5-hydroxytryptamine (5-HT) and 5-hydroxyindole acetic acid (5-HIAA) were measured in different rat brain regions after amino acid decarboxylase inhibition. The aromatic L-amino acid decarboxylase inhibitor (m-Hydroxybenzyl-hydrazine, NSD 1015®, EGA-Chemie, West Germany) were injected intraperitoneally (100 mg per kg body weight), 30 min prior to decapitation resulting in an inhibition of the conversion from 5-HTP to 5-HT. Analyses were made in 7 different brain regions, dissected immediately after decapitation; at the

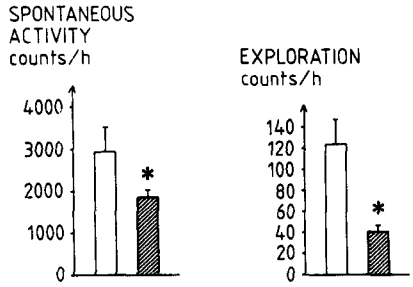

FIG. 1. Open field tests with measurements of spontaneous activity (left) and exploratory nose-poke (right) at the same time but recorded separately. Number of counts (i.e. broken photobeams) during one hour is registered. * denotes $p<0.01$ compared to the sham group, which was set as a criterion for significance. Open column: Sham (n=6); striped column: PCS (n=12).

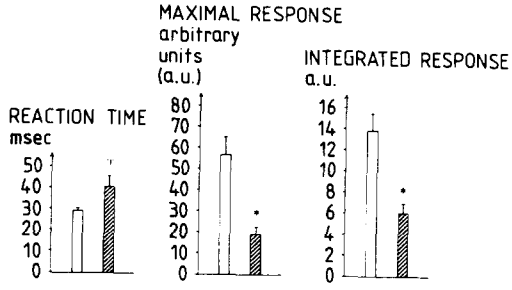

FIG. 2. Unconditioned startle response to somatonsensory stimulation by a 0.5 mA, 100 msec unscrambled electrical pulse given in a grid floor. For each animal a mean value of five successful trials was taken. Presented are means and SEMs for the two groups in reaction time in msec (left), maximal amplitude of the response in arbitrary units (height of the curve peak) (middle), and integrated response in arbitrary units (area under curve) (right) which is, depending on the reaction time (latency to respond to stimulus), the maximal response, but also takes the total duration of the response into consideration. † denotes $p<0.05$ compared to the sham group, * denotes $p<0.01$ compared to the sham group. Open column: Sham (n=6); striped column: PCS (n=12).

same time blood from the neck wound was taken for plasma ammonia analysis by an enzymatic technique [4]. The major brain regions studied were: (1) cortex, (2) striatum, (3) septum-hippocampus, (4) diencephalon, (5) mesencephalon-pons, (6) proximal medulla spinalis, and (7) distal medulla spinalis. Brain pieces were immediately frozen and stored in liquid nitrogen until the chemical analyses were performed.

Indolederivates. The analysis of 5-HTP, 5-HT, and 5-HIAA was performed with high performance liquid chromatography (HPLC) with electrochemical detection. The tissue specimens were homogenized in 5.0 ml 0.4 M perchloric acid. The homogenate was centrifuged at 34,000 × g for 10 min, and the supernatant was then filtered. For each analysis of 5-HTP, 5-HT, and 5-HIAA performed in the same run, 0.5 ml of this filtrate was placed in the automatic injector (see Apparatus) after addition of 100 ng α-methyl-5-HT as an internal standard. Ten μl 5% L-Cystein solution was added to the filtrate as an antioxidant for 5-HIAA (for methodological details, see [2]).

Apparatus. A Waters Intelligent Sample Processor (WISP 710 B, Water Associates Inc., Milford, MA) was used together with a high pressure pump (LKB 2150 HPLC Pump, Bromma, Sweden). A guardcolumn (LC-8 Supelguard, Supelco Inc., Bellefonte, PA) 2 cm × 0.30 cm i.d. was used, followed by a column (LC-8 DB) 15 cm × 0.46 cm i.d. and both were packed with 3 μm Supelcosil. The electrochemical detection was made by a thin-layer amperometric detector (Model LC-4, Bioanalytical Syst. Inc., West Lafayette, IN), and the working electrode (glassy carbon, Model TL-5A) potential was maintained at 0.67 V vs. Ag/AgCl (3 M NaCl) reference electrode. Results were calculated and presented on an integrator (SP 4270, Spectra-Physics, San Jose, CA) connected to the system. For comparison a standard containing 100 ng/ml of 5-HTP, 5-HT, α-methyl-5-HT, and 5-HIAA was used. The mobile phase contained 4.0 g methane sulphonic acid and 3.0 g orthophosphoric acid per litre of 3% methanol, and the pH was adjusted to 2.5 by 10 M NaOH. The flow rate was 1.0 ml/min. All chromatographic runs were performed at ambient temperature.

Statistical Analysis

Comparison between the PCS and sham-operated rats on each separate behavioral test and in each brain region for 5-hydroxyindoles was carried out by calculating the Student's *t*-value. For the water maze behavior results, a 2-way analysis of variance was used. Pearson Product Moment Correlation Coefficient was used for multiple correlation analysis, and a criterion for significance of $p<0.01$ was set for 2 tail *t*-tests.

RESULTS

There was a decrease in body weight in PCS rats compared to sham-operated animals (PCS (n=12) 258±5 g vs. Sham (n=6) 231±8 g; mean±SEM; $p<0.01$). Ammonia levels in plasma showed an increase ($p<0.01$) in PCS rats (225±12 μmoles/l) compared to sham operative controls (111±7 μmoles/l). All PCS animals had plasma ammonia levels above 200 μmoles/l.

Behavior

In the automated open field boxes, the PCS rats showed reduced levels of both locomotor activity and nose-poke exploration (Fig. 1).

In the somatosensory reactivity the startle response to an electric foot shock was measured with 3 parameters. All of them were impaired in the PCS rats compared to sham-operated controls (Fig. 2). The latency to react was prolonged in the shunted group. The maximum amplitude of the response was reduced, and the total integrated response (reaction integral) also showed a decrease in shunted animals compared to sham-operated controls.

In the water maze test the acquisition swim latencies for shunted and sham-operated rats are shown in Fig. 3. Statistical analysis could not demonstrate any differences between the groups, as illustrated in the curves. On the last block of 4 trials when the platform had been removed, there was no difference observed between the number of times rats from either group passed over the location where this platform had been (mean±SEM, PCS (n=12) 14.9±1.7, Sham (n=6)

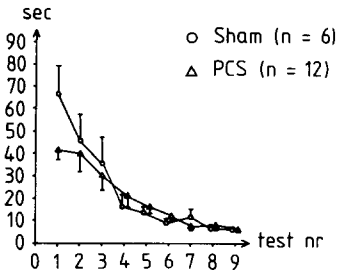

FIG. 3. Swim latencies (in seconds) after introduction of the rats to the water maze pool. The abscissa shows the first 36 consecutive trials in blocks of 4 trials (4 × 9 = 36). Means of swim latencies was taken for each rat after every fourth trial. Presented are the means and SEMs for sham and PCS groups. There was no difference between groups using a 2-way analysis of variance.

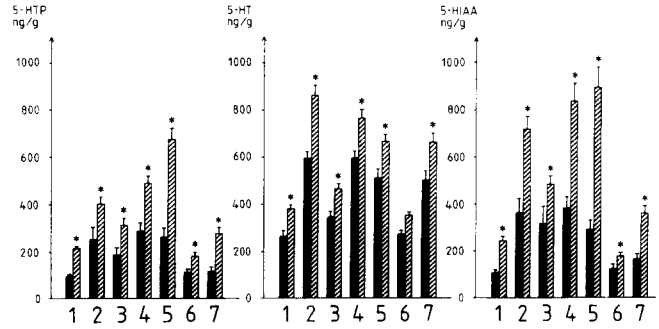

FIG. 4. Brain indoles 3 weeks after operation (means and SEMs). 5-Hydroxytryptophan (left), 5-hydroxytryptamine (middle), and 5-hydroxyindole acetic acid (right) in 7 rat brain regions. Solid columns: Sham (n=6) and striped columns: PCS (n=12). All indoles were measured as concentrations in ng/g brain tissue. * denotes $p<0.01$ compared to the sham group, which was set as a criterion for significance. 1=cortex, 2=striatum, 3=septum-hippocampus, 4=diencephalon, 5=mesencephalon-pons, 6=prox. medulla, 7=dist. medulla.

16.5±1.4). These data do not indicate impaired spatial learning or memory after PCS in the rat.

No statistical differences were found between the two groups on any of the behavioral measures used to assess motor coordination. Within and between the groups variance was small.

In the step-through passive avoidance test the shunted and sham-operated groups did not differ on the training day with respect to step-through latencies (less than 10 sec as a mean value for both groups). On the testing day most subjects did step-through within the 120 sec allowed. The mean time for the PCS rats was 41±9 sec, and 38±17 sec for the sham-operated controls. No difference in long-term memory between the groups was established.

Brain Indoles

Three weeks after PCS there was a marked increase compared to sham-operated animals in the accumulation of 5-HTP in the brain following decarboxylase inhibition (Fig. 4). In all investigated brain regions the 5-HTP levels were higher in PCS rats than in sham-operated controls. This feature was most prominent in the mesencephalic-pontine region. The concentrations of 5-HT and 5-HIAA were also increased in all brain regions in the PCS rats. This increment was more pronounced for 5-HIAA than for 5-HT (Fig. 4). The difference in 5-HIAA concentrations between PCS and sham-operated rats was most pronounced in the mesencephalic-pontine region, thus in the same region as the highest 5-HTP accumulation was observed.

Intercorrelations

Multiple correlation analyses were performed in order to compare, in the mesencephalon-pons region of the individual PCS animals, the extent to which the different behavioral impairments were correlated with the increased synthesis rate of the brain indoles. This analysis gave no support for correlation between neurochemical alterations and behavioral deficits.

DISCUSSION

The use of the PCS rat as a model for chronic liver insufficiency with encephalopathy in man has prompted detailed mapping of the neurochemical and the behavioral abnormalities in the rat. Thus, behavioral alterations like hypoactivity, decreased startle to various stimuli, and disrupted sleep patterns and circadian rhythms have been described in PCS rats [1, 2, 6, 7, 17, 21, 22, 25].

Increased plasma ammonia levels and disturbed weight gain as observed in the rats in the present experiment are established criteria for patency of the shunt, which is vital for the use of the model [8].

The current investigation showed that rats with PCS, 3 weeks after operation, exhibited impaired open field acitivty (i.e., spontaneous locomotion and exploratory behavior) when individually tested, as well as decreased somatosensory reactions. The hypoactivity is in agreement with previous reports [2, 17, 21]. Normal activity after PCS has also been demonstrated [18]. A possible explanation for this difference might be that the PCS rats in the latter experiment were tested 6–7 months after surgery when development of blood vessel collaterals to the liver can take place resulting in a normalization of the liver blood flow [6]. Even increased spontaneous activity has been demonstrated in PCS rats [6]. In this study, however, increased activity was shown during the light period when rats normally sleep, and a different method for the activity measurements was used.

Decreased startle to both tactile, auditory [25] and foot-shock stimuli [7,22] has also been reported. Our investigation using foot-shock stimuli showed that the inhibition of the somatosensory reactivity was present in all measured parameters; latency to respond, maximal response, and the integrated response, which also considers the duration of the response. The fact that different stimuli result in similar startle responses indicate that this behavioral alteration is due to disturbances in the central nervous system, rather than in peripheral nerves or receptors.

The motor coordination did not differ between PCS rats and sham-operated controls. Thus, the front paw strength (the wire suspension test) of the PCS rats was equivalent to that of sham animals. This not only demonstrates unaltered coordination between the groups, but also provides evidence that the open field impairments of the PCS rats is not only

due to a more rapid muscular exhaustion of those animals.

The lack of differences in swim latencies in the water maze performance between the two experimental groups demonstrated that PCS rats mimicked the sham controls in spatial learning and memory for this task. Furthermore, no differences between the two groups were observed for long-term memory functions as illustrated in the step-through passive avoidance test. These findings partly coincide with previous observations of unaltered cognitive functions of PCS rats when exposed to different alleyway configurations in a maze test [17].

Neurochemical data from this experiment demonstrated increased 5-hydroxyindole synthesis rate in all regions of the central nervous system in PCS rats with behavioral alterations. This is in agreement with results from other authors [9, 21, 22, 25]. By using decarboxylase inhibition the increase in synthesis of those compounds is more obvious (for details see [2]).

Serotonin as a central nervous system neurotransmitter has been implicated in a wide variety of mammalian behavioral processes, such as arousal, locomotion, feeding, sex, aggression, and nociception [13]. Topographically, the serotonergic cell bodies in the medullary raphe nuclei project both rostrally (i.e., cortical and hypothalamic regions) and caudally (spinal) [3]. The wide spread distribution of serotonin containing nerve fibres supports the hypothesis that brain serotonin may exert a general modulatory effect on behavior, rather than mediating a specific behavioral process.

An explanation for the altered startle response could be that large elevation of serotonin produces a change in escape thresholds in the direction of hypoalgesia [20]. An interesting observation, however, is that intraventricular injection of serotonin to the forebrain depresses acoustic startle, while serotonin injection intrathecally to the spinal cord gives the opposite result [10]. The authors suggest that this excitatory effect is mediated by a facilitation of the response of the lower motor neurons to the excitatory volley initiated by the startle stimulus. Still, the most common reports are those of an inverse action of the startle responds compared with the brain serotonin levels [11, 22, 26]. Our findings are in agreement with those observations. Divergent results on the correlation between brain serotonin and startle response are, however, reported. Significant reduction of brain 5-HT and 5-HIAA levels after treatment with parachloroamphetamine did not influence the foot-shock sensitivity in either PCS or control rats [7].

The decreased open field activity could also be caused by serotonergic dysfunction, especially since this behavior is the result of complex integration involving forebrain systems. The overall altered behavior in PCS rats could, however, also be due to functional changes in other neurotransmitter systems, or the result of intoxication of ammonia, mercaptans, etc. [15]. Furthermore, reports of increased 5-HT and 5-HIAA levels in rat brains 6–7 months after PCS have been made. Those animals did not exhibit altered spontaneous activity [18].

In this study rats with PCS displayed increased brain indole metabolism and a selective behavioral syndrome involving decreased generalized motor activity and sensory-motor integration, with no changes in motor coordination or cognitive abilities.

However, no correlation between behavioral impairment and brain serotonin metabolism was established. This does not exclude a role for altered serotonin synthesis in PSE, but other biochemical alterations of catecholamines, neuropeptides, false neurotransmitters, etc., may interact with serotonin to give a full complex picture of PSE.

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