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Strain Specific Alterations in Hippocampal Cholinergic Function Following Acute Footshock[1]

DENNIS E. SCHMIDT, DAVID O. COOPER[2] AND ROBERT J. BARRETT

Department of Pharmacology, Vanderbilt University, Nashville, TN 37232
and
Tennessee Neuropsychiatric Institute, 1501 Murfreesboro Road, Nashville, TN 37217

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SCHMIDT, D. E., D. O. COOPER AND R. J. BARRETT. *Strain specific alterations in hippocampal cholinergic function following acute footshock.* PHARMAC. BIOCHEM. BEHAV. 12(2) 277–280, 1980.—Previous studies have shown that differences between Z-M and F-344 rats in active avoidance acquisition are due to variation in stress-induced motor response, rather than to differences in general learning ability. Thus F-344 rats, which become active when exposed to shock, are more likely to make avoidance responses and learn to associate an active response with ommision of shock than are Z-M rats, which reduce their activity in response to shock. The hypothesis that cholinergic neurons in the hippocampus contribute to the stress-induced motor suppression seen in Z-M rats derives from pharmacologic studies employing micro-injection of cholinergic antagonists. The present studies further investigate this hypothesis through assessment of acetylcholine turnover and high affinity choline uptake in discrete brain regions of Z-M and F-344 rats. Increases in cholinergic function in the dorsal hippocampus were observed in Z-M, but not F-344 rats following acute footshock. Cholinergic alterations were not seen in the ventral hippocampus or striatum of Z-M rats. The strain specific alterations in dorsal hippocampal cholinergic function correlate with documented motor suppression in Z-M rats and motor activation in F-344 rats during acute shock, and suggest that this cholinergic system mediates a suppressive behavioral response to environmental stress.

Cholinergic Footshock Hippocampus Strain differences Septo-hippocampal pathway
Avoidance conditioning High affinity choline uptake

THERE is considerable evidence for a cholinergic pathway in the central nervous system (CNS) which interacts with behavioral performance of rats in conditioned shock avoidance paradigms. Administration of cholinergic antagonists (e.g. scopolamine) facilities acquisition of two-way active avoidance tasks [2, 4, 19, 29], but disrupts acquisition of passive avoidance responses [15, 24, 25, 27]. This effect of anti-cholinergics has been attributed to an attenuation of a cholinergically mediated, stress-induced motor suppression which is compatible with learning of passive but not active avoidance responses [2].

Moreover, recent studies indicate that this phenomenon is a strain dependent, genetically determined variable [28]. F-344 rats, which display motor activation during acute footshock acquire active avoidance tasks exceptionally well. In contrast, Z-M rats acquire active avoidance poorly due to interferences by shock-induced motor suppression. Other strains of rats (CDF, Holtzman) show intermediate degrees of activation and learning [3,28]. Additional studies have clearly demonstrated that since both the F-344 and Z-M strains learn equally well *where* to run, the differential performance of Z-M and F-344 rats in active avoidance conditioning is dependent upon the extent of motor activation, rather than on general learning ability [2]. This conclusion is further substantiated by the fact that in passive avoidance paradigms, where motor activation is inappropriate to the task, the performance of Z-M rats is significantly better than that of F-344 rats [2]. Finally, the heritable nature of these behavioral differences between Z-M and F-344 rats has been verified through hybridizing and cross fostering studies [28].

The primary anatomical locus of this cholinergic suppressive system appears to be the dorsal hippocampus. Microinjection of scopolamine into the dorsal hippocampus produces a facilitation of active avoidance equivalent to that seen with systemic scopolamine administration [20]. Similar alterations were not observed following microinjection of

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scopolamine into the ventral hippocampus [20] or caudate nucleus [23]. Furthermore, electrolytic lesioning of the dorsal hippocampus produces facilitation of active avoidance which has been attributed to an attenuation of shock-induced behavioral suppression [10, 13, 16, 17, 26].

Finally, additional support for existence of a cholinergic suppressive pathway in the hippocampus arises from developmental data. Behavioral suppression in various test situations has been shown to be age dependent. Rats less than 20 days old are inferior to adults in passive avoidance learning [11,36], display higher rates of spontaneous locomotor activity than adults [21], and have more difficulty withholding responses in spontaneous alternation tasks [1,9]. Furthermore, scopolamine begins to interfere with passive avoidance learning in rats at approximately 20 days of age, but not prior to this time [12]. Similarly, scopolamine potentiates amphetamine induced behavioral arousal in 20–25 day old rats, but not in those 15 days of age [6]. These data have been attributed to the functional development of a hippocampal cholinergic system in the rat between 14 and 21 days of age [1, 9, 21].

To date, however, no direct biochemical measurements of this hypothetical cholinergic pathway in response to avoidance paradigms have been reported. The present studies were therefore designed to test the hypothesis that cholinergic neurons in the dorsal hippocampus should be preferentially activated in those rat strains which undergo stress-induced motor suppression when compared to those strains which exhibit stress-induced behavioral activation. Sodium dependent high affinity choline uptake (HACU) in synaptosomal preparations and regional ACh turnover were therefore measured in Z-M and F-344 rats following acute footshock. These biochemical parameters, when taken together, accurately reflect in vivo cholinergic neuronal activity.

METHOD

Animals

Sprague-Dawley (ZM) rats were obtained from Zivic-Miller Laboratories, Allison Park, PA. F-344 rats were supplied by Harlan Industries, Cumberland, IN. Rats, all male, were 55–65 days old upon arrival, and were housed in groups of 4–5 for one week prior to use. Food and water were supplied ad lib and lighting followed on a 0700 on–1900 off cycle.

Footshock Procedure

Rats were placed in a rectangular compartment (approximately 10×25 cm) with a floor made of 25 parallel stainless steel grids. Inescapable shock (1.75 mA; 1.5 sec) was delivered through the grid floor every 30 sec for 20 min. Rats were sacrificed immediately following a single shock session. Control rats were sacrificed directly from their home cages in alternation with their shocked counterparts.

High Affinity Choline Uptake

Following decapitation, hippocampi and striata were rapidly dissected over ice. Hippocampi were further dissected to separate the dorsal 1/3 from the ventral 2/3. All brain regions were then placed in 5 ml cold isotonic sucrose (0.32 M). Following homogenization (glass-teflon pestle), samples were centrifuged at 1000×g for 10 min to remove cellular debris. Supernatants were then re-centrifuged for 20 min at 22,000×g. The resulting synaptosomal pellets were resuspended in 1 ml cold isotonic sucrose.

The rate of high affinity choline uptake was measured by a modification [7] of the method Kuhar *et al.* [18]. Briefly, 100 μl of the synaptosomal suspension was incubated with 0.9 ml of sodium phosphate or sodium free buffer (37°C) containing 10^{-6} M (0.4 μC) ^{3}H-choline iodide (TRK-179, Amersham Searle) in 1.5 ml Beckman microfuge tubes. Following 4 min incubation, the reaction was terminated and the pellets re-isolated by 3 min centrifugation in a Beckman microfuge. The supernatant was discarded by careful decantation and the pellets are surface washed twice with 1.0 ml cold isotonic saline. The bottom 2 cm of the tube was then cut and placed directly in 10 ml of ACS counting fluid (Amersham-Searle) for scintillation counting. Under these conditions the rate of uptake was linear between 2 and 8 min. High affinity choline uptake (i.e. corrected for sodium-free low affinity background) was calculated on the basis of choline acetyltransferase activity. This method has been shown to be superior to HACU determined on the basis of total protein [7].

Acetylcholine Turnover

Relative acetylcholine turnover was determined by measuring the rate of decline in acetylcholine levels following intraventricular administration of hemicholinium-3 (HC-3) [33]. Rats were given 20 μg HC-3, placed in the shock apparatus for 20 min, and immediately sacrificed by head-focussed microwave irradiation [32]. Control rats were given HC-3 only and were similarly sacrificed 20 min later in alternation with their shock counterparts. Acetylcholine levels were measured by pyrolysis gas chromatography as previously described [34].

Statistical Analysis

Data were analyzed by the Student t distribution with the criterion of significance set at $p<0.05$.

RESULTS

Acute footshock produced a significant increase in high affinity choline uptake in the dorsal hippocampus of Z-M rats. Under identical experimental conditions, dorsal hippocampal HACU was not altered in F-344 rats. Figure 1 illustrates typical replications of this experiment.

These results were further corroborated by measurement of relative acetylcholine turnover under similar experimental conditions (Fig. 2). Z-M rats display significant increases in hippocampal acetylcholine turnover rates following acute footshock, while this parameter remains unaltered in F-344 rats. These data, which demonstrate cholinergic alterations in the dorsal hippocampus in Z-M, but not F-344 rats provide strong evidence for the existence of a genetically determined biochemical reaction to stress.

The regional specificity of stress-induced cholinergic alterations was also established. Within the same experiments as those reported in Fig. 1, changes in HACU observed in the dorsal hippocampus of Z-M rats were not observed in either the ventral hippocampus or the striatum of either strain. In fact, a small but consistent decrease in HACU was seen in the ventral hippocampus of Z-M rats.

DISCUSSION

The studies presented in this manuscript provide direct biochemical evidence of a specific increase in cholinergic function in the dorsal hippocampus of Z-M rats following

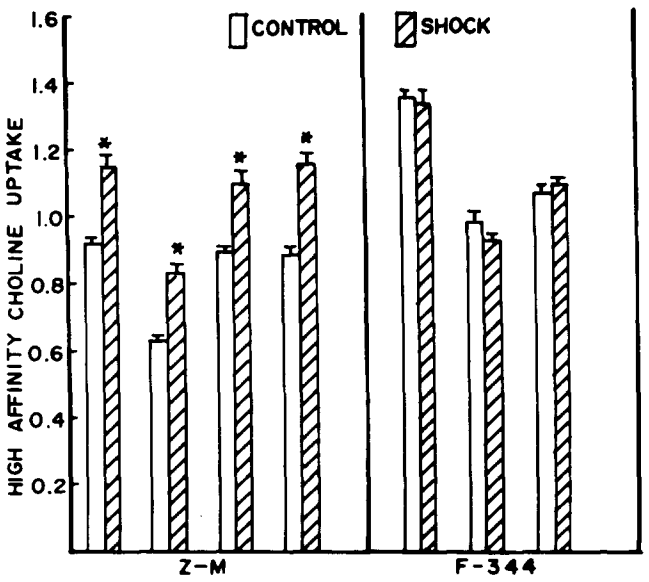

FIG. 1. Effect of acute footshock on Na^+-dependent high affinity choline uptake in dorsal hippocampus of ZM and F-344 rats. Results represent the mean ± SEM of individual experiments. Number of animals per experiment, from left to right, were n=8, n=8, n=8, n=10, n=8, n=8, n=10. Choline uptake values are expressed as pmol choline/nmol ACh synthesized/min. *=$p<0.05$ by Student t test (two-tailed).

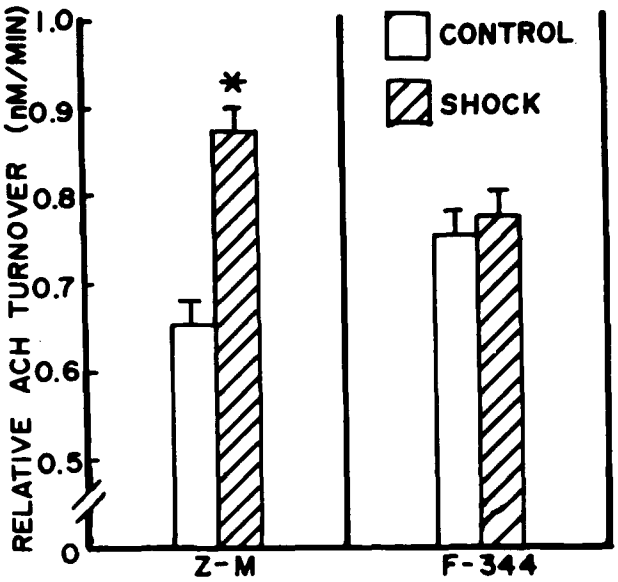

FIG. 2. Effect of acute footshock on the relative rate of acetylcholine turnover in the dorsal hippocampus of ZM and F-344 rats. Results represent the mean ± SEM (n=12 Ss group). *=$p<0.05$ by Student t test (two-tailed).

shock-stress. Significantly, under identical experimental conditions no cholinergic changes were seen in dorsal hippocampus of F-344 rats. These cholinergic changes correlate with the occurrence of stress-induced motor suppression in Z-M rats and the lack of such suppression in F-344 rats. Furthermore, the cholinergic alterations observed in the dorsal hippocampus of Z-M rats do not represent a general activation of cholinergic systems in brain during stress, as can be seen by the lack of change in HACU in either the ventral hippocampus or striatum. These data, therefore, provides biochemical evidence for the existence of a cholinergic system in the dorsal hippocampus which is responsible for stress-induced behavioral suppression. Furthermore, it corroborates previous evidence that facilitation of active avoidance acquisition in Z-M rats occurs following microinjection of scopolamine into the dorsal, but not ventral hippocampus [20] or caudate nucleus [23] and is consistent with a report that the level of HACU in hippocampus varies inversely with locomotor activity [5].

Histochemical [22,35] and autoradiographic [30] studies indicate that the cell bodies for hippocampal cholinergic neurons are primarily located in the medial septal nucleus. Behavioral evidence further indicates that this septal-hippocampal pathway does regulate the level of responses to various behavioral paradigms. For example, medial septal lesions result in hyper-reactivity [31], improved active avoidance performance [8] and impaired acquisition of passive avoidance tasks [14]. There are numerous and complex neuronal inputs into the septal region involving a number of different neurotransmitter systems and the relationships of these transmitter influences are poorly understood (for review see[37]). Such a multiplicity of influence is however, both necessary and useful because it allows various neurotransmitter systems to modulate behavioral responses to numerous different types of environmental stimuli. The consistent correlation of the behavioral and cholinergic changes seen in these experiments however, suggest that the septo-hippocampal cholinergic pathway may represent a final common output from these various modulatory influences. As such, the further investigation of changes in the septo-hippocampal cholinergic pathway during acute stress, the nature of the neurotransmitter systems involved in producing the changes at the level of the septal nucleus, and the heritability and strain dependent differences in stress induced alterations should provide valuable information on a major behavioral control system.

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