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BRIEF COMMUNICATION

Increase in the Brain Regional Depolarization-Dependent Ca^{2+} Uptake in Rats Preferring Ethanol[1]

PETER H. WU,[2] THERESA FAN AND CLAUDIO A. NARANJO

Clinical Pharmacology and Neurology Programs, Addiction Research Foundation Clinical Institute, Toronto, Ontario, Canada

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WU, P. H., T. FAN AND C. A. NARANJO. *Increase in the brain regional depolarization-dependent Ca^{2+} uptake in rats preferring ethanol.* PHARMACOL BIOCHEM BEHAV 27(2) 355–357, 1987.—The depolarization-dependent Ca^{2+} uptake system has been suggested to be involved in the release of transmitter and synaptic facilitation. It can be employed as an effective probe to study neurotransmission. Although ethanol has been shown to inhibit or facilitate neurotransmission very little is known about the intrinsic activity of neurotransmission in ethanol-preferring rats. Using the depolarization-dependent Ca^{2+} uptake system, we demonstrated that synaptic neurotransmission is more active in animals with moderate and high preference for ethanol. Results suggest that there are intrinsic differences in the brain regional neurotransmission among rats showing different degrees of preference for ethanol.

Depolarization-dependent Ca^{2+} uptake Neurotransmission Ethanol preference

SYNAPTIC transmission consists of the pre-synaptic release of a transmitter and a post-synaptic response. When a depolarization arrives at pre-synaptic terminals, a transient influx of Ca^{2+} and a transient release of transmitter have been observed [7]. The effects of ethanol treatment on neurotransmission have been thoroughly investigated (see review, [13]). In most experiments, acute ethanol inhibits transmitter release [3] and chronic ethanol inhibits [15] or stimulates [10,11] transmitter release in various brain regions. Although studies have been done to investigate the effects of ethanol on neurotransmission, relatively fewer studies have investigated neurotransmission in animals showing a high preference for ethanol. We used depolarization-dependent Ca^{2+} uptake as a general index for activity of transmitter release to investigate the neurotransmission in rats showing low preference, moderate preference and high preference for ethanol.

METHOD

Male Wistar rats weighing 200–250 g were housed in stainless steel cages in a room regulated for temperature (22°C), humidity (55%) and 12-hour day-night cycle (7:00 p.m. to 7:00 a.m. darkness). The cages were equipped with two 100 ml calibrated glass Richter tubes to deliver fluid. Five pellets per animal per day of standard rat chow were given at 1600 hr. Animals were given a free choice between tap water and an ethanol solution (2–6%) in a two-bottle preference paradigm. Richter tubes were emptied, rinsed and refilled with fresh solution daily, with fluid consumption levels determined (at 1500 hr). Each day, upon each refilled fluid presentation, the position of the two drinking vessels was exchanged in order to avoid bias in fluid consumption generated by a position preference. Ethanol solutions were presented to the animals in 2% increments every fourth day from 2% to 6% (v/v).

Three different groups of rats were selected during a two week period: those who drank less than 1 g/kg/day (ethanol/body weight) or did not drink at all were grouped as low preferring, those who drank an average of 2 g/kg were moderate preferring and those who drank over 4 g/kg of ethanol were selected as high preferring. The rats were given tap water only for 2 weeks after the determination of preference. On experimental days, animals were sacrificed at 10:00 a.m. Brain regions were dissected according to the method described by Glowinski and Iversen [5] and were homogenized

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[2]Requests for reprints should be addressed to Dr. P. H. Wu, Clinical Pharmacology Program, Addiction Research Foundation, 33 Russell Street, Toronto, Ontario M5S 2S1, Canada.

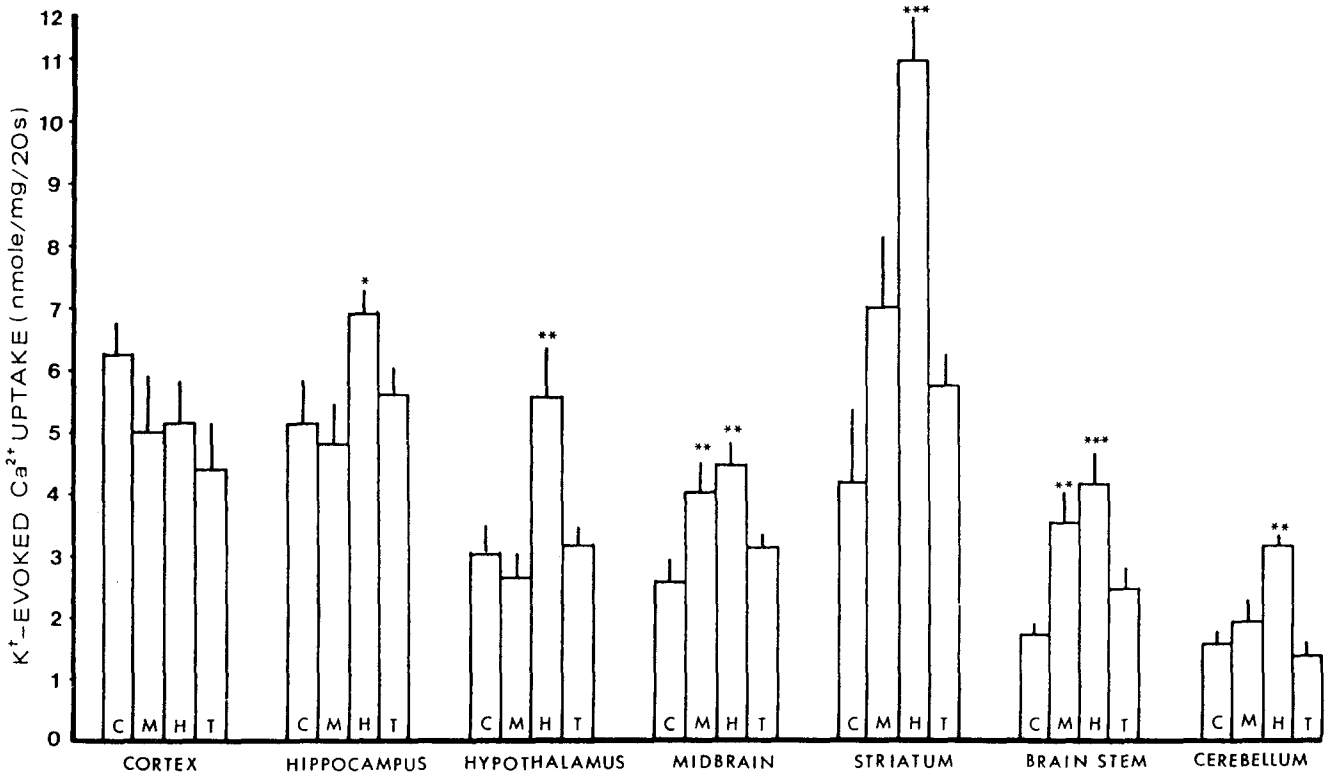

FIG. 1. K^+-evoked Ca^{2+} uptake in brain regions of ethanol preferring rats. Rats were selected according to their preference for ethanol in a free choice paradigm. Rats which drank an average of 4.4 g/kg (ethanol/body weight) were indicated as high preference, whereas rats which drank an average of 2.0 g/kg and less than 1 g/kg were classified as moderate and low preference respectively. Rat brain regions were then used for ^{45}Ca-uptake. Results are mean±S.E.M. of 5–9 determinations of two separate experiments. C=low preference; M=moderate preference for ethanol; H=high preference for ethanol; T=6 g/kg, 2 days ethanol treated animals. Student's t-test was used. *$0.025<p<0.05$ ($t=2.262$, n=10); **$0.0005<p<0.005$ ($t=3.298$, n=13); ***$p<0.0005$ ($t=5.608$, n=14).

in 0.32 M sucrose solution. The P_2 fraction (crude mitochondrial fraction) was prepared according to Gray and Whittaker's method [6]. The K^+-evoked ^{45}Ca-uptake was determined according to Wu *et al.* [14]. Briefly, P_2 fraction from brain regions was resuspended in the incubation buffer (132 mM NaCl, 5 mM KCl, 1.3 mM $MgCl_2$, 1.2 mM NaH_2PO_4, 1.0 mM $CaCl_2$, 1.0 mM glucose and 20 mM Tris-HCl, gassed with 95% O_2 and 5% CO_2 to reach pH 7.5 at 37°C). One hundred μl of P_2 suspension (0.25 to 0.5 mg protein) were pre-incubated with 855 μl buffer solution at 37°C for 20 min. Forty-five μl of $^{45}CaCl_2$ (Amersham, Oakville, Ontario, specific activity 1 μCi/1.0 μmole) in 1 M KCl solution were added, and reaction was continued for 20 sec. For the non-depolarized ^{45}Ca-uptake, 45 μl of $^{45}CaCl_2$ in 1 M NaCl were used. The ^{45}Ca-uptake was stopped by adding 5 ml of EGTA solution (120 mM NaCl, 5 mM KCl, 5 mM EGTA and Tris-HCl to a final pH of 7.5). Following the filtration of P_2 particles and washing them with 2×5 ml of incubating buffer on a GF/B filter, the radioactivity associated with P_2 particles was extracted and counted in 8 ml Aquasol (New England Nuclear). The counting efficiency was 92%.

RESULTS

The amount of Ca^{2+} taken up by the P_2 fraction in 45 mM K^+ over the 5 mM K^+ medium was defined as depolarization-dependent Ca^{2+} uptake (ΔK, K^+-evoked Ca^{2+} uptake) and it was found that ΔK reached maximum at 20 sec incubation [14]. Figure 1 shows that in the ethanol low preferring rat brain, cortex, hippocampus and striatum, there are high levels of K^+-evoked Ca^{2+} uptake. The ΔK of brain regions in the ethanol moderate preferring rats also shows higher levels of activity in cortex, hippocampus and caudate (striatum). However, the brain regional distribution of ΔK in ethanol high preferring rats was striatum > hippocampus≥cortex. When rats were treated with 6 g/kg/day ethanol for two days, the brain region of ΔK was similar in cortex, hippocampus and striatum. The apparent change in ΔK distribution in brain regions was due to a significant increase in ΔK in selective brain regions of the high preferring animal. The hippocampal ΔK was significantly higher ($0.025<p<0.05$) in high preferring, but was not significantly different in moderate and low preferring rats. A significant increase in hypothalamic ΔK was also found in high preferring animals ($0.0005\leq p\leq 0.005$). Higher levels of ΔK were found in midbrain and brainstem of the moderate preferring rats.

Among the 7 brain regions examined, hippocampus, hypothalamus, midbrain, caudate, brainstem and cerebellum, all showed significantly higher ΔK in the high preferring rats. The striatum showed a profound increase in ΔK activity. When rats were treated with 6 g/kg ethanol for 2 days, no significant changes in ΔK activity in these 7 brain regions were observed.

DISCUSSION

There are genotypic variations in ethanol's effects on brain dopamine turnover in C57BL and BALB/c mice [4,8] suggesting a genotypic variation in sensitivity of CNS neurotransmission to ethanol. Since ΔK (depolarization-dependent Ca^{2+} uptake) has been shown to directly or indirectly trigger the release of transmitter substances [7], measuring ΔK will be a valid expression of transmitter release [1] and neurotransmission. The ΔK values in different brain regions found in our study are similar to those reported by Leslie *et al.* [9]. The increases in ΔK values in midbrain and brainstem of moderate ethanol preferring rats suggest an enhanced neurotransmitter release in these two brain regions which are known to be involved in conditioning [2]. The results cannot preclude the possibility that the enhancement of the reticular activating system resulted from prior exposure to ethanol as a conditioning stimulus. In the high ethanol preferring rats, the ΔK in all brain regions except cerebral cortex were elevated, suggesting that these animals may have an overall increase in basal level of neurotransmission. The increase in ΔK of the moderate and high ethanol preferring animals cannot be explained by the chronic or residual effects of ethanol alone, since chronic ethanol treatment for 2 days did not change ΔK in all brain regions, and decreased ΔK in cortex, hippocampus and hypothalamus after 8.5 days of treatment (unpublished observation). Therefore, the observed brain regional increases in the depolarization-dependent Ca^{2+} uptake in the moderate and high ethanol preferring rats may contribute to reinforcement exerted at the cellular level [12].

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