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Long-term daily access to alcohol alters dopamine-related synthesis and signaling proteins in the rat striatum

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ABSTRACT

Chronic alcohol exposure can adversely affect neuronal morphology, synaptic architecture and associated neuroplasticity. However, the effects of moderate levels of long-term alcohol intake on the brain are a matter of debate. The current study used 2-DE (two-dimensional gel electrophoresis) proteomics to examine proteomic changes in the striatum of male Wistar rats after 8 months of continuous access to a standard off-the-shelf beer in their home cages. Alcohol intake under group-housed conditions during this time was around 3–4 g/kg/day, a level below that known to induce physical dependence in rats. After 8 months of access rats were euthanased and 2-DE proteomic analysis of the striatum was conducted. A total of 28 striatal proteins were significantly altered in the beer drinking rats relative to controls. Strikingly, many of these were dopamine (DA)-related proteins, including tyrosine hydroxylase (an enzyme of DA biosynthesis), pyridoxal phosphate phosphatase (a co-enzyme in DA biosynthesis), DA and cAMP regulating phosphoprotein (a regulator of DA receptors and transporters), protein phosphatase 1 (a signaling protein) and nitric oxide synthase (which modulates DA uptake). Selected protein expression changes were verified using Western blotting. We conclude that long-term moderate alcohol consumption is associated with substantial alterations in the rat striatal proteome, particularly with regard to dopaminergic signaling pathways. This provides potentially important evidence of major neuroadaptations in dopamine systems with daily alcohol consumption at relatively modest levels.

1. Introduction

Habitual excess alcohol intake affects the biochemistry of brain, but the effect of moderate consumption is unclear. Moderate ethanol intake, sometimes defined as two standard drinks or approximately 27 g ethanol/day in humans (Dufour, 1999), may indeed have beneficial cardiovascular effects and promote longevity (Brügger-Andersen et al., 2009). However, there remains the possibility that moderate daily drinking can lead to subtle neuroadaptations that may be difficult to detect in standard brain imaging or post mortem neuropathological investigations (Eckardt et al., 1998).

Heavy drinking shrinks the brain, reduces the functional efficiency of neurons and glial cells, and cause major disruptions to the biochemical processes necessary for cellular homeostasis (Harper, 2009; Moonat et al., 2010). Human brain imaging studies indicate that alcohol intake causes a marked reduction in whole brain glucose metabolism and modified brain resource allocation (Koob, 2006; Volkow et al., 2008). At the same time, neuroadaptations in motivation-related brain pathways contribute to the addictive process, whereby alcohol becomes compulsively sought out and its consumption becomes a habitual process that is resistant to reprogramming (Guerri and Pascual, 2010; Moonat et al., 2010).

The dorsal and ventral striatum, along with connected prefrontal and limbic regions, are considered to have major roles in such addiction-related neuroadaptations (for recent review see Chen et al., 2011). The neurotransmitter dopamine (DA) is present in high concentrations in these regions and is strongly implicated in

Abbreviations: AC, adenyl cyclase; AR, aldose reductase; cGMP, cyclic guanylyl monophosphate; CREB, cyclic adenosine monophosphate (cAMP) responsive element binding protein; DA, dopamine; DARPP-32, DA and cAMP regulated phosphoprotein; DHAP, converting dihydroxyacetone phosphate; eNOS, endothelial nitric oxide synthase; G3PDH, glycerol-3-phosphate dehydrogenase; GBP, guanine trisphosphate binding protein; H_2O_2, hydrogen peroxide; HAD, high alcohol drinking; MG, methylglyoxal; MST, 3-mercaptopyruvate sulfur transferase; NADHD, NADH dehydrogenase; NO, nitric oxide; NOS, nitric oxide synthase; NP, alcohol-non-preferring; P, alcohol preferring; PP1, protein phosphatase-1; PPP, pentose phosphate pathway; PPPase, pyridoxal phosphate phosphatase; ROS, reactive oxygen species; SOD, superoxide dismutase; TCA, tricarboxylic acid cycle; TH, tyrosine hydroxylase; TPP, tyrosine phosphatase; V-ATPase, vacuolar ATPase.

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the neurobiology of addictive behaviors. Ethanol acutely stimulates DA release leading to increased extracellular DA in striatal synapses (Maldonado-Devincci et al., 2010; Weiss et al., 1993). However, chronic alcohol intake may lead to neuroadaptations associated with lowered basal dopaminergic tone leading to an increased vulnerability to the reinforcing effects of alcohol (Chen et al., 2011; Volkow et al., 2007; Weiss et al., 1996). Rat strains specially bred for high levels of alcohol consumption – the alcohol preferring (P) and high alcohol drinking (HAD) strains – have lower basal DA and DA metabolites in the cerebral cortex and dorsal and ventral striatum (McBride et al., 1993; Strother et al., 2005). Similarly, human data suggest a link between low dopaminergic tone in the striatum and increased vulnerability to alcoholic overindulgence (Connor et al., 2008; Volkow et al., 2007). Overall, these findings suggest that moderate and prolonged levels of alcohol intake may alter the biochemistry of striatal regions, with lowered DA function in this region perhaps both a cause and a consequence of excessive alcohol intake. Whether this occurs with moderate alcohol intake is unknown, although there is some suggestive evidence from animal studies. For example, rats administered ethanol (3 g/kg/day for 8 weeks) did not display any tolerance or dependence to the drug, but exhibited alterations in DA release and receptor sensitivity, and related intracellular signaling processes in the striatum (Battaini et al., 1998).

Proteomics can be a powerful approach to examine global changes in protein expression resulting from drug and alcohol exposure. Recent studies document alcohol-induced alterations in regional protein expression in *post-mortem* alcoholic human brains. Areas studied include the prefrontal cortex (Alexander-Kaufman et al., 2006), corpus callosum (Kashem et al., 2009a, 2008, 2007) and hippocampus (Matsuda-Matsumoto et al., 2007). Animal studies have similarly shown altered protein expression in the nucleus accumbens of P, alcohol-non-preferring (NP) and Wistar rats after five daily injections of a relatively low dose of ethanol (1 g/kg) (McBride et al., 2009). We have reported subtle alterations in protein expression in the hippocampus of adolescent rats given 4 weeks of *ad libitum* access to standard off-the-shelf beers (Hargreaves et al., 2009b). Access to beer promotes moderate to high levels of alcohol consumption in non-selected lines of rats (Hargreaves et al., 2009a, 2011; McGregor and Gallate, 2004). In the present study we continued our use of the "beer model" of alcohol consumption and examined protein changes in the striatum of rats given much longer (8 months) daily access to alcohol.

2. Experimental procedures

2.1. Subjects

Male albino Wistar rats from the in-house breeding colony in the School of Psychology at the University of Sydney were used in the experiment. The rats were housed in large group cages with $n = 4$ rats per cage in a reversed light-dark cycle colony (12-hour light: 12-hour dark, lights off at 7 am) with *ad libitum* access to water and food at all times.

A total of 24 rats were initially allocated to two equal groups ($n = 12$ each): a "beer" group (mean body weight 290 g) and a "control" group (mean body weight 285 g). Approval for the experiments was obtained from the University of Sydney Animal Ethics Committee and all procedures adhered to Australian National Guidelines for the Use of Animals for Scientific Purposes.

2.2. Procedure

Rats assigned in the "beer" group were allowed to consume beer under *ad libitum* conditions. Using a "step-up" procedure (Hargreaves et al., 2009a,b, 2011), these rats were initially given Birell's "Ultra Light Premium" near-beer (Coopers, Ltd. South Australia), a near-beer beverage that contains 0.44% ethanol (22 calories/100 ml). Over a 20 day interval, a steadily increasing proportion of "Tooheys New" beer (Lion Nathan, Australia), a common off-the-shelf beer in Australia (4.6% ethanol v/v, 38 calories/100 ml) was mixed into the Birell's "Ultra Light Premium" until by day 20 only the "Tooheys New" beer was present. In total, the rats were exposed to beer of 0.44%, 1%, 2%, 3% and 4% alcohol content for 4 days each, followed by "Toohey's New" for the rest of the experiment.

Daily access to beer continued in the home cage for a total of 8 months. A two bottle paradigm was used with beer and water always available to the rats. The side of presentation of beer and water was randomly alternated and bottles were weighed daily, with fresh solutions being presented every 2 days. During the sixth month of the experiment, every rat was individually housed for a 7 day period to allow more accurate information to be collected regarding individual food, water and beer intake. After these individual measurements rats were returned to group housing for a further 2 months during which beer presentation in a group housed environment continued as usual.

At the end of 8 months the rats were killed via decapitation without anaesthetic. The 24 brains were removed and the whole striatum rapidly dissected and stored at −80 °C. The striatal tissue of the top 5 consumers of beer during the 7 days of individual consumption, and of six body weight matched control rats, were selected for proteomic analysis.

2.3. Protein extraction from rat brain tissue for proteomics

The protocol for protein extraction and gel analysis was as previously described (Hargreaves et al., 2009b; Kashem et al., 2008, 2009b,c). Briefly, 50 mg of tissue was homogenized in 1 ml of buffer A (7 M urea, 2 M thiourea, 1% C7bZO (a zwitterionic detergent) and 40 mM Tris–HCl) followed by sonication three times at 70% intensity/100% cycle in 10 s bursts. The crude protein extracts were collected after centrifugation at 16,000*g* for 20 min at 15 °C. The supernatant was reduced and alkylated in 5 mM tributyl phosphine and 10 mM acrylamide monomer at room temperature for 2 h. The reaction was quenched using DTT (dithiothreitol) to 10 mM. Protein precipitations were obtained by adding acetone (five times of extract volume) and citric acid (20 mg) followed by centrifugation at 2500*g* for 15 min at 15 °C. The pellet was air dried and resuspended in 0.5 ml of buffer B (7 M urea, 2 M thiourea and 1% C7bZO). Finally, the extract was aliquoted and stored at −80 °C before isoelectric focusing.

2.4. Two-dimensional gel electrophoresis (2-DE)

The protein concentration of extracts was determined using the Bradford method, with human brain protein used as a standard (Bradford, 1976). Consumables for gel electrophoresis were obtained from Proteome Systems Ltd. (Australia) and IPG (immobilized pH gradient) strips were from Biorad Lab Pty Ltd. (Australia). 2-DE was as previously described (Hargreaves et al., 2009b; Kashem et al., 2008, 2009b; Matsuda-Matsumoto et al., 2007). To reduce overlapping protein expression, we used a narrow range of IPG strips (pH 4–7) that includes approximately 80% of cytosolic proteins (Pozzi Mucelli et al., 2006). IPG strips (11 cm) were re-hydrated with samples containing 400 μg of protein for 6 h at room temperature. The re-hydrated strips were focused for a total of 120 kV h. IPG strips were equilibrated using equilibration buffer followed by loading onto SDS–PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) gels (8–16%, 10 × 15 cm) for second dimension (30 mA/gel, 25 °C for 110 min). The gel was fixed in a solution consisting of 25% (v/v) methanol