Alcohol and Caffeine: The Perfect Storm

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Although it is widely believed that caffeine antagonizes the intoxicating effects of alcohol, the molecular mechanisms underlying their interaction are incompletely understood. It is known that both caffeine and alcohol alter adenosine neurotransmission, but the relationship is complex, and may be dose dependent. In this article, we review the available literature on combining caffeine and alcohol. Ethical constraints prohibit laboratory studies that would mimic the high levels of alcohol intoxication achieved by many young people in real-world settings, with or without the addition of caffeine. We propose a possible neurochemical mechanism for the increase in alcohol consumption and alcohol-related consequences that have been observed in persons who simultaneously consume caffeine. Caffeine is a nonselective adenosine receptor antagonist. During acute alcohol intake, caffeine antagonizes the "unwanted" effects of alcohol by blocking the adenosine A_1 receptors that mediate alcohol's somnogenic and ataxic effects. The A_1 receptor–mediated "unwanted" anxiogenic effects of caffeine may be ameliorated by alcohol-induced increase in the extracellular concentration of adenosine. Moreover, by means of interactions between adenosine A_{2A} and dopamine D_2 receptors, caffeine-mediated blockade of adenosine A_{2A} receptors can potentiate the effects of alcohol-induced dopamine release. Chronic alcohol intake decreases adenosine tone. Caffeine may provide a "treatment" for the withdrawal effects of alcohol by blocking the effects of upregulated A_1 receptors. Finally, blockade of A_{2A} receptors by caffeine may contribute to the reinforcing effects of alcohol.

Introduction

ON OCTOBER 30, 1991, the eastern seaboard of the United States was struck by "a perfect storm," so called because its destructive power rose from an unprecedented set of circumstances: an ice-cold high-pressure system moving south from Canada, a low-pressure system over the Great Lakes moving east toward Nova Scotia, and a late season hurricane moving north from the tropics, ironically called "Grace." The mixture of alcohol, caffeinated energy drinks, and youth has been described as another "perfect storm," because it combines "high pressure" (the central nervous system stimulant caffeine), "low pressure" (the central nervous system depressant alcohol), and the "tropical hurricane" of youth, a period of life characterized by risk taking, independence seeking, and experimentation.[1] In this article, we review the available literature on combining caffeine and alcohol, explain the neurochemical basis for their interaction, and propose a possible mechanism for the "storm" aftermath: greater alcohol consumption and an increase in serious alcohol-related consequences.

Human Metabolism of Caffeine

Caffeine absorption is rapid and complete in humans, with 99% of orally ingested caffeine absorbed from the digestive tract within 45 minutes.[2] Absorption is not modified by gender, genetics, liver disease, or the ingestion of drugs or alcohol.[3] Caffeine crosses all biological membranes and is distributed in all body fluids.[3] Peak plasma concentrations are observed within 1–2 hours following a single oral dose of caffeine (4 mg/kg).[3] Neither caffeine nor its metabolites accumulate in the organs or tissues of the body. In adults, 1%–2% of ingested caffeine is excreted directly in the urine; 98% of caffeine is metabolized by the cytochrome P450 system of the liver into three active metabolites: paraxanthine, theobromine, and theophylline.[3] The isozyme CYP1A2 is responsible for 90% of caffeine clearance.[3]

Numerous factors modify caffeine clearance

- In infants, the immaturity of hepatic enzyme systems impairs the elimination of caffeine, in comparison to adults.

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- Liver disease, especially cirrhosis, significantly reduces clearance of caffeine.
- The half-life of caffeine is significantly prolonged in women taking oral contraceptives.[2] The menstrual cycle does not significantly alter the pharmacokinetics of caffeine in healthy eumenorrheic women.[4]
- Pregnancy increases the half-life of caffeine, in part due to decreased CYP1A2 activity. Caffeine crosses the placenta, but moderate caffeine consumption (less than 200 mg/day) does not appear to increase risk for spontaneous abortion, preterm birth, low birth weight, or congenital malformations.[5]
- Cigarette smoking nearly doubles the rate of caffeine metabolism, by increasing liver enzyme activity.[2]
- Quinolone antibiotics (e.g., ciprofloxacin and norfloxacin) decrease the metabolism of caffeine in a dose-dependent manner, most likely by inhibiting the activity of CYP1A2 isozymes.[6]
- Alcohol, grapefruit juice, and cruciferous vegetables prolong the half-life of caffeine.[3,6]

Human Metabolism of Alcohol

Orally ingested alcohol is rapidly absorbed into the human bloodstream from the stomach and small intestine and distributes into total body water. On an empty stomach, peak blood alcohol levels occur about 30 minutes following the oral ingestion of alcohol.[7] For ingested liquids, the principal determinant of the gastric emptying rate is volume; large volumes empty at an exponentially faster rate than small volumes.[8] Because alcohol absorption occurs more rapidly in the small intestine, delays in gastric emptying decrease the rate of alcohol absorption. Food in the gastrointestinal tract slows alcohol absorption; the higher the dietary fat content of a meal, the longer the absorption process takes.[7]

A small amount of alcohol is metabolized in the stomach by the enzyme alcohol dehydrogenase (ADH). Compared with men, women have lower activity of ADH in their stomachs, causing a larger proportion of ingested alcohol to reach the bloodstream.[7] Alcohol is primarily metabolized by the liver, at a steady rate independent of how much alcohol has been consumed. The rate of liver metabolism, typically between 15 and 20 mg% per hour, varies somewhat among individuals, depending on the amount and efficiency of one's liver enzymes, genetic variation in the enzymes, and medication use.[7]

Blood alcohol concentration in humans is therefore determined by a number of factors, including individual rates of absorption and metabolism, gender, body weight, percentage of body water, use of medications, the rate of drinking, and concurrent consumption of food.

Challenges in Human Research on Caffeine/Alcohol

Caffeine is the most studied drug in history, but confounding variables cause difficulty with the interpretation of research on its human health effects. The caffeine in foods and beverages may be difficult to quantify. The caffeine content of coffee cannot be easily estimated from the reported intake of coffee, because different methods of roasting, grinding, and brewing affect the amount of caffeine.[9] A "single serving" of coffee may range from 4 to 16 ounces (120–

480 mL); "an energy drink" may be an 1 ounce (30 mL) "shot" or a 23.5 ounce (705 mL) can. Different brands of energy drinks of the same size contain different amounts of caffeine. Aversive side effects and a reduction in perceived benefits generally limit the doses of caffeine used by the general population. In human experimental studies, the amount of caffeine consumed is typically moderate, making it difficult to establish a dose-response relationship for adverse effects. Caffeinated foods and beverages may contain other pharmacologically active substances, making it difficult to isolate the effects of caffeine. Even experiments using standardized dosages with humans in laboratory settings are confounded by individual variation in the rate of caffeine metabolism, differences in sensitivity to caffeine, and lifestyle habits. Research on women has been limited by concerns about possible reproductive risks. Finally, many original studies are confounded by failure to account for withdrawal and withdrawal reversal effects in human subjects.

Laboratory studies in human subjects have not established the safety of coingestion of caffeine with high levels of alcohol. Individual expectancies, the timing of dose administration, individual variability in both alcohol tolerance and caffeine sensitivity, and differences in the types of cognitive and performance tests used as outcomes make it very difficult to interpret human health effects.[10] Moreover, ethical constraints prohibit human experiments that would reproduce the manner of consumption and the level of intoxication typically achieved by young adults in real-world situations, making it difficult to extrapolate laboratory findings.

Research on Human Consumption of Caffeine and Alcohol: Prevalence Studies

Malinauskas *et al.* surveyed 492 college students regarding patterns of energy drink use.[11] The consumption of energy drinks "with alcohol while partying" was reported by 57% of women and 50% of men. Among students who drank three or more energy drinks in a given situation, 49% stated that they did so to mix with alcohol while partying.[11] In a survey of 450 Italian medical students by Oteri *et al.*, 56.9% reported energy drink consumption.[12] Of the total sample, 48.4% mixed energy drink and alcohol; 36% of those who reported ever combining energy drink and alcohol had done so on more than three occasions in the previous month.[12] Attila and Çakir investigated the frequency of energy drink usage among 439 fourth-year college students in Turkey.[13] Among current users, 37.2% reported that they mixed energy drinks with alcoholic beverages. In a survey of 72 male energy drink users at a Halifax university, 76% of participants reported having mixed energy drink and alcohol.[14] Students drank significantly more alcohol on occasions when they also consumed energy drinks (8.6 drinks vs. 4.6 drinks; $p = 0.016$).[14]

Research on Human Consumption of Caffeine and Alcohol: Association with Alcohol-Related Problems

Energy drink consumption was positively associated with a problem behavior syndrome in a survey of 602 undergraduates by Miller, who found that this relationship was significantly moderated by race.[15] Frequent consumers of energy drinks reported drinking alcohol and having alcohol-related problems more than twice as often as less-frequent energy

drink consumers or nonconsumers. Frequent energy drink consumption was positively associated with alcohol problems in White students, but not in Black students.[15] Among college students who reported past 30-day drinking, 24% consumed alcohol mixed with energy drinks, in a 2008 study by O'Brien et al.[16] The consumption of alcohol mixed with energy drink was strongly associated with high-risk drinking behavior, including increased binge drinking and more frequent episodes of weekly drunkenness. Even after adjusting for the increased alcohol consumed, college students who drank caffeinated alcoholic beverages had significantly higher prevalence of serious alcohol-related consequences, including being sufficiently sick or injured as a result of drinking to seek medical attention, being taken advantage of sexually, and riding with a drunken driver.

Thombs et al. conducted an event-level analysis of 802 exiting patrons in an U.S. college bar district.[17] Drinkers who reported mixing alcohol with energy drink had a threefold risk of being legally intoxicated (breath alcohol content ≥0.08 g/210 L, the legal limit for operating a motor vehicle in the United States for drivers aged 21 years or older), and a fourfold increased odds of reporting the intention to drive a motor vehicle when leaving the bar district, compared with drinkers who reported consuming alcohol alone.

In a longitudinal study conducted by Arria et al., the annual weighted prevalence of caffeinated energy drink usage among 1060 undergraduates at a large public university in the United States was $22.6\%_{wt}$ in the second year and $36.5\%_{wt}$ in the third year.[18] Compared with nonenergy drink users, students who used energy drinks drank alcohol more frequently and in higher quantities. Prospectively, energy drink users were significantly more likely than nonenergy drink users to initiate nonmedical use of prescription stimulants and prescription analgesics, even after adjustment for demographics, sensation-seeking personality score, caffeine consumption, and prior use of the drug of interest.

Arria et al. explored the associations between caffeinated energy drink usage, alcohol-use patterns, and alcohol-related consequences in 1097 fourth-year college students.[19] Students who reported occasional or monthly energy drink consumption were classified as "low-frequency" users (52.5% of respondents). Students who reported consuming energy drinks on weekly, almost-daily, or daily basis were classified as "high-frequency" users (13% of respondents). Compared with low-frequency energy drink users, high-frequency energy drink users were significantly more likely to have gotten drunk at an early age. Independent of demographics, high-frequency users drank alcohol more frequently and in higher quantities, and were twice as likely to meet Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV) criteria for alcohol dependence, compared with low-frequency users.[20]

Research on the Human Consumption of Caffeine and Alcohol: Laboratory Studies

Azcona et al. used a double-blind placebo-controlled crossover study of eight healthy male volunteers to evaluate the psychomotor and subjective effects of alcohol and caffeine.[21] In four experimental sessions, participants were given placebo, alcohol (0.8 g/kg body weight), caffeine (400 mg), or alcohol (0.8 g/kg body weight) plus caffeine (400 mg). Plasma concentrations of alcohol and caffeine were measured at baseline and at eight intervals after drug intake. Subjects were requested to abstain from alcohol, coffee, tea, or cola for 24 hours before and throughout the experimental session. Psychomotor performance was measured by critical flicker fusion (a measure of cortical arousal), simple reaction time (SRT), and a tapping (reflex) test. Visual evoked potentials and two assessments of subjective mood were also utilized. Alcohol consumption significantly increased SRT and decreased amplitude of the evoked potentials. Caffeine decreased SRT and increased the amplitude of the evoked potentials. In this experiment, the profiles of placebo and the combination of alcohol plus caffeine were not significantly different from results of either of the two substances alone. The addition of caffeine to alcohol did not significantly change subjective feelings of depression, anxiety, or drunkenness.

Ferreira et al. tested physiologic indicators (heart rate, blood pressure, respiratory exchange rate, oxygen uptake, and ventilatory threshold), biochemical variables (glucose, lactate, insulin, cortisol, ACTH, dopamine, norepinephrine, and epinephrine), blood alcohol levels, and the performance of participants on a maximal effort on a bicycle ergometer.[22] All subjects at baseline were "moderate" users of alcohol and "moderate" consumers of energy drinks; individual differences in consumption were not controlled. In a double-blind study over four sessions, 14 male volunteers were given weight-based doses of alcohol (1.0 g/kg), energy drink (3.57 mL/kg Red Bull®), energy drink plus alcohol, and a control beverage (water). No significant differences in physiologic and biochemical parameters were observed between the alcohol and the alcohol plus energy drink sessions. The performance in the maximal effort test following the ingestion of energy drink plus alcohol was similar to that observed with alcohol alone.

Ferreira et al. gave weight-based doses of alcohol alone (either 0.6 or 1.0 g/kg), a caffeinated energy drink alone, or alcohol plus energy drink to 26 healthy male volunteers in three separate experimental sessions.[23] Participants were "similar" in their baseline use of alcohol and energy drinks. Researchers measured breath alcohol concentrations, motor coordination (using the Grooved Peg-board test), and visual reaction time. Subjective intoxication was evaluated using a visual analog scale of somatic symptoms. Compared with the ingestion of alcohol alone, the consumption of energy drink plus alcohol significantly reduced subjective drunkenness, but did not significantly ameliorate alcohol-induced deficits in motor coordination and visual reaction time. The addition of energy drink did not alter breath alcohol concentration in persons who consumed alcohol.

Marczinski and Fillmore evaluated dual-task interference and psychologic refractory periods in 12 adult volunteers using a double-blinded within-subject design that crossed two doses of alcohol (0.0 and 0.65 g/kg) with three doses of caffeine (0.0, 2.0, and 4.0 mg/kg).[24] Participants (six men and six women) were instructed to fast for 4 hours, abstain from caffeine for 8 hours, and abstain from alcohol for 24 hours, prior to the study session. Alcohol significantly impaired information processing, increasing the psychologic refractory period needed to complete a second task performed in close proximity to a first task. Response accuracy to the

second task was also impaired. Coadministration of caffeine antagonized alcohol-induced impairment of the psychologic refractory period, but it had no antagonizing effect on alcohol-induced impairment of accuracy. Participants in Marczinski's study reported reduced subjective intoxication in response to caffeine coadministration, despite their performance impairment.

Curry and Stasio studied a global measure of neuropsychological functioning in 27 nonsmoking women, using a double-blinded placebo-controlled model in which participants consumed 16 ounces of a nonalcoholic, noncaffeinated beverage (*Diet 7-Up*), a caffeinated energy drink alone (*Monster Green*, 160 mg caffeine/16 ounces), or a caffeinated 6% alcoholic malt beverage (*Sparks Orange*, 87 mg of caffeine).[25] Participants were asked to abstain from caffeine use for 1 hour prior to the assessments. Researchers used the Repeatable Battery for the Assessment of Neuropsychological Status to evaluate five cognitive domains: immediate memory, delayed memory, visuospatial/constructional, attention, and language. Body weight was used to calculate an estimated blood alcohol level. Compared with the energy drink group, visuospatial construction and language scores were significantly decreased in the group who consumed caffeinated alcohol. Both *Monster* and *Sparks* contained proprietary amounts of guarana.

In response to anecdotal evidence that links sudden cardiac death with the combination of energy drinks, alcohol, and exercise, Wiklund *et al.* examined 10 healthy volunteers who consumed energy drinks (three 250 mL cans of *RedBull*®), energy drink plus alcohol (0.4 g of ethanol per kilogram of body weight), or no drink, 30 minutes prior to maximal effort on a bicycle ergometer.[26] Researchers required "at least a 1-week washout period" between experimental sessions. No clinically significant dysrhythmias were observed, but postexercise, recovery in heart rate and heart rate variability was slower for participants who consumed energy drink plus alcohol, compared with those who consumed energy drink alone. The authors suggest that blunted cardiac autonomic modulation after exercise may increase risk of dysrhythmia for predisposed individuals.

In a randomized trial by Howland *et al.*, nonsmoking, nondependent, heavy-episodic adult drinkers (*n* = 127) received caffeinated beer, noncaffeinated beer, caffeinated nonalcoholic beer, or noncaffeinated nonalcoholic beer using a 2×2 between-groups model.[27] All participants consumed ≥1 and ≤7 caffeinated beverages daily. Alcoholic beverage administration was targeted to achieve breath alcohol concentration of 0.12 g%. A 30-minute simulated driving test and the Psychomotor Vigilance Task (PVT) were administered; participants were asked to estimate their own blood alcohol levels using a previously developed questionnaire. Alcohol intoxication resulted in more variability in speed and lateral movement, increased crashes, and significantly impaired reaction times. Caffeine did not significantly alter alcohol-impaired driving or PVT performance. Researchers found no difference in self-estimated blood alcohol concentration between the participants who received caffeinated beer and those who received noncaffeinated beer.

Marczinski *et al.* investigated the effect of alcohol plus energy drink on response activation and inhibition in 56 adults (28 men and 28 women), using a cued go/no go task.[28] Participants were required to abstain from caffeine for 8 hours and alcohol for 24 hours prior to the experiment. In a single laboratory session, participants were randomly assigned to receive alcohol (0.5 g/kg of 40% ABV vodka), energy drink (3.57 mL/kg of *RedBull*®), alcohol plus energy drink, or placebo (3.57 mL/kg of a decaffeinated soft drink). Alcohol significantly impaired both response execution and response inhibition. The consumption of energy drink mixed with alcohol antagonized the alcohol-induced impairment of response execution, but not alcohol-induced impairment of response inhibition. Individuals who consumed energy drinks mixed with alcohol reported increased levels of stimulation compared with those who drank alcohol alone, but the addition of energy drink did not significantly alter the drinker's subjective feelings of intoxication or the drinker's perception of ability to drive.

Research on the Effects of Caffeine and Alcohol: Animal Studies

Gulick and Gould used male C57BL/6J mice to test the effect of caffeine on alcohol-induced changes in anxiety, locomotion, and plus-maze discriminative avoidance.[29] The plus-maze resembles a plus sign, with two opposing enclosed arms and two opposing open arms. An aversive arm delivers 75-W light and 85-Db white noise when a mouse is in the arm. The plus-maze model measures learning (as percent time spent in aversive enclosed arm versus percent time in the nonaversive enclosed arm; increased time in the aversive arm and decreased time in the nonaversive arm = decreased learning) as well as anxiety (as percent time spent in the open arms; increased time in the open arms = decreased anxiety). In this experiment, alcohol decreased anxiety and learning in a dose-dependent fashion; caffeine increased anxiety and decreased locomotion and learning in a dose-dependent fashion. Caffeine did not reverse alcohol-induced learning deficits, but a high dose of alcohol (1.4 g/kg) was noted to block the anxiogenic effect of caffeine.

A study by El Yacoubi *et al.* compared the hypnotic effects of alcohol following the administration of caffeine (25 mg/kg) in $A_{2A}R$ WT and $A_{2A}R$ KO mice.[30] At lower doses, alcohol acts as a central nervous system depressant in mice, causing sedation and incoordination. Higher doses of alcohol significantly impair consciousness, causing a "coma-like" state that is measured by loss of the righting reflex (an animal's failure to correct its position when lying on its back). Adenosine receptor deficient mutant mice ($A_{2A}R$ KO) were less sensitive to alcohol-induced loss of righting reflex, a finding that suggests that the A_{2A} receptor is involved in mediating the behavioral effects elicited by intoxicating doses of alcohol.

Kunin *et al.* observed that a narrow dose range of caffeine facilitated an increase in alcohol drinking behavior in free-feeding laboratory rats.[31] Animals that were given an intraperitoneal injection of caffeine (5 mg/kg) demonstrated a dose-related increase in the consumption of 8% and 10% alcohol, but those given 10 mg/kg of caffeine did not differ in their alcohol consumption from placebo (saline-treated) animals. A second experiment assessed the effect of caffeine on the maintenance of established alcohol consumption. In rats that had been acclimatized to increasing concentrations of alcohol, the administration of caffeine enhanced alcohol consumption at a dosage of 5 mg/kg, but animals treated

with 2.5 and 10 mg/kg caffeine did not differ from saline-treated animals. Researchers offered two possible hypotheses to explain this phenomenon: that the stimulant effect of caffeine encouraged "self-medication" with alcohol, or that caffeine sensitized the rats to alcohol's reinforcing effects.

Mechanisms of the Pharmacological Interactions of Alcohol and Caffeine

Both in humans and the experimental animal, caffeine produces the same qualitative pharmacological effects as classical psychostimulants, such as cocaine and amphetamine: an increase in motor activity, arousal effects, and reinforcing effects.[32,33] It is important to point out that caffeine has a weaker reinforcing efficacy than classical psychostimulants.[32] Thus, caffeine users often fulfill the criteria for drug dependence, but not for drug abuse, established by the DSM-IV.[20] This seems to be mostly due to the fact that, different to classical psychostimulants, there is a little window between the "wanted" psychostimulant effects and the "unwanted" effects of caffeine, specially anxiety.

On the other hand, alcohol is psychodepressant and has somnogenic, anxiolytic, and motor-depressant and motor-impairing (ataxic) properties, but also experimentally proven reinforcing effects.[34] Similarly to what happens with caffeine, there is a relatively little window between the "wanted" anxiolytic and reinforcing effects and the "unwanted" somnogenic and ataxic effects of alcohol. Just by comparing the pharmacological effects of caffeine and alcohol, and without entering into the mechanistic aspects, it becomes obvious that alcohol–caffeine could be a desired drug combination, since they could mutually counteract their unwanted effects. Thus, the arousal properties of caffeine could compensate the somnogenic effects of alcohol, while the anxiolytic effects of alcohol could compensate the anxiogenic effects of caffeine. In fact, as we will be describing with more detail in the following section, there is experimental evidence supporting these pharmacological interactions.[26,35–37] But, what it becomes really intriguing is that, in fact, there is a common target for most "unwanted" and some of the "wanted" effects of caffeine and alcohol: adenosine neurotransmission.

Adenosine as a Mediator of the Psychostimulant Effects of Caffeine

Caffeine is a nonselective competitive adenosine receptor antagonist and produces its psychostimulant effects by counteracting the tonic effects of endogenous adenosine on central adenosine receptors. This depends largely on the ability of adenosine to modulate the function of multiple central ascending neurotransmitter systems, which are involved in motor activation and reward (dopaminergic systems) and arousal effects (cholinergic, noradrenergic, histaminergic, and orexinergic systems). Among the four cloned adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3 receptors), A_1 and A_{2A} receptors are the ones predominantly expressed in the brain. Caffeine has similar *in vitro* affinities for A_1, A_{2A}, and A_{2B} receptors and much lower affinity for A_3 receptor.[32,33,38] A_1 and A_{2A} receptors are the preferential targets for caffeine in the brain, since physiological extracellular levels of adenosine are sufficient to occupy and, therefore, stimulate A_1 and A_{2A} receptors. On the other hand, A_{2B} receptors have a lower affinity for adenosine and they are only activated by pathologically high extracellular levels of adenosine.[32,33,38] A_1 receptors are widely expressed in the brain, including the striatum, while A_{2A} receptors are highly concentrated in the striatum.[32,33,38]

Striatal A_1 and A_{2A} receptors seem to underlie the motor-activating and reinforcing effects of caffeine, which depend on its ability to release the strong functional brake that adenosine imposes to the ascending dopaminergic systems.[33] There are two ascending dopaminergic systems, which originate in the substantia nigra pars compacta and the ventral tegmental area and innervate the dorsal and ventral striatum, respectively. Striatal A_{2A} receptors modulate dopamine neurotransmission by establishing direct interactions with dopamine D_2 receptors, forming A_{2A}-D_2 receptor heteromers. In these heteromers, stimulation of A_{2A} receptors blocks D_2 receptor function.[39] In the ventral striatum, interactions between A_{2A} and D_2 receptors seem to play an important role in the reward-related behaviors.[40,41] The same as D_2 receptor antagonists, A_{2A} receptor agonists elevate brain stimulation reward threshold, indicating that adenosine, via A_{2A} receptors, may inhibit central reward processes.[42] Further, stimulation of striatal presynaptic A_1 receptors inhibits while its blockade facilitates dopamine release by glutamate-dependent and glutamate-independent mechanisms.[43] By targeting A_1 receptors in striatal glutamatergic terminals and A_1 receptors in striatal dopamine terminals (presynaptic brake), caffeine induces glutamate-dependent and glutamate-independent release of dopamine. These presynaptic effects of caffeine are potentiated by the release of the postsynaptic brake imposed by antagonistic interactions in the striatal A_{2A}-D_2 heteromers.[33]

An important amount of evidence indicates that adenosine is a mediator of sleepiness following prolonged wakefulness. Initial evidence came from pharmacological studies that show the sleep-inducing effects of systemic or intracerebral administration of adenosine and adenosine receptor agonists.[44] It is now generally believed that a direct A_1 receptor–mediated modulation of the corticopetal basal forebrain system and an indirect A_{2A} receptor–mediated modulation of the hypothalamic histaminergic systems are the main mechanisms by which adenosine exerts its sleep-promoting effects.[33,45] However, it is important to point out that a possible additional contribution comes from A_1 receptors localized in the nuclei of origin of the histaminergic, orexinergic, and noradrenergic arousal systems.[33] Arousing effects of caffeine depend on the blockade of multiple inhibitory mechanisms that adenosine, as an endogenous sleep-promoting substance, exerts on the multiply interconnected ascending arousal systems.[33]

Adenosine Mechanisms in the Acute Pharmacological Effects of Alcohol

The pharmacological effects of alcohol are multiple, since it targets many neurotransmitter receptors and ion channels, involving a multitude of neurotransmitter systems in widespread regions of the brain.[34] Among those systems, alcohol is known to potentiate GABAergic neurotransmission by facilitating $GABA_A$ receptor–mediated currents by direct and indirect mechanisms as well as by promoting GABA release.[34,46] In addition, alcohol inhibits glutamatergic
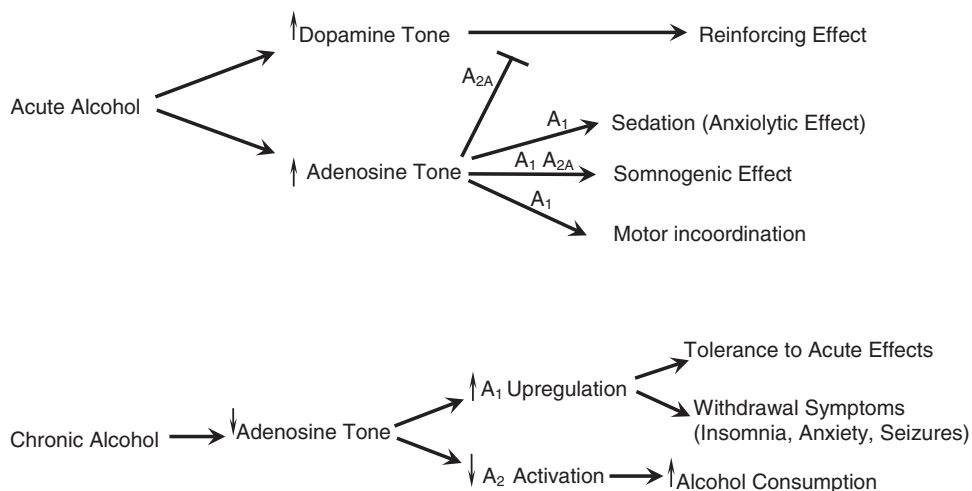
FIG. 1. Adenosine mechanisms involved in the pharmacological interactions of caffeine and alcohol. Acute consumption of alcohol induces an increase in the dopamine tone that is responsible for its reinforcing effects. At the same time, alcohol induces an increase in the adenosine tone, which opposes its dopamine-mediated reinforcing effects by means of antagonistic striatal A_{2A}-D_2 receptor interactions, and which is responsible for the nonwanted effects of alcohol (such as motor incoordination and somnogenic effects). Caffeine, by antagonizing the acute effects of alcohol, provides a unique tool to enhance the reinforcing effects and attenuate the nonwanted effects of alcohol. Under conditions of chronic alcohol consumption, there is a reduced adenosine tone, which is associated with a decreased activation of A_{2A} receptors, which might increase dopaminergic neuro-transmission and, therefore, contribute to the increased alcohol consumption, and with upregulation of A_1 receptors, which might be involved in the tolerance to the acute effects of alcohol and also to the withdrawal symptoms (see text).

neurotransmission by acting on ionotropic glutamate receptors, especially by attenuating N-methyl-D-asparate receptor function.[34,47] Obviously, both the facilitation of GABAergic neurotransmission and the inhibition of glutamatergic neurotransmission are most probably involved in the central depressant effects of alcohol. Further, alcohol modulates dopamine neurotransmission by directly affecting the firing activity of dopamine neurons in the VTA.[48] The mechanisms underlying this effect of alcohol are not completely elucidated, but it seems to involve a direct effect on a subtype of potassium channel that regulates the excitability of VTA neurons, as well as indirect effects through modulation of inputs to the VTA.[48] It is widely believed that alcohol-induced increase in VTA neuronal firing, with the resulting increase of dopamine release in the ventral striatum, mediates the reinforcing effects of alcohol.[49,50] It is important to point out that recent evidence indicates that acetaldehyde formed from alcohol in the brain or in the periphery participates in the effects of alcohol on VTA neurons.[51]

Apart from GABA, glutamate, and dopamine, an important amount of experimental data shows that adenosine is a main neurotransmitter involved in the acute and chronic pharmacological effects of alcohol (Fig. 1).[35–37,52] Pioneering studies by Dar *et al.* showed that the adenosine uptake blocker dipyridamole potentiated, whereas the adenosine receptor antagonist theophylline reduced, the acute somnogenic and ataxic effects of alcohol in mice.[53] Further, they also reported that the potentiating effect of dipyridamole was not present with chronic alcohol treatment.[53] Finally, they found that alcohol withdrawal was associated with a significant decrease in A_1 receptor density in the brain.[53] Later on, Proctor and Dunwiddie found that the different sensitivity to the motor-depressant effects of alcohol of two mouse lines correlated with the sensitivity for the

motor-depressant effects of an A_1 receptor agonist and the motor-activating effect of theophylline.[54]

After those initial studies, clear data have been obtained indicating that adenosine, by acting on A_1 receptors, is a key mediator of the acute ataxic, somnogenic, and anxiolytic effects of alcohol (Fig. 1). The extensive work by Dar and coworkers supports the participation of adenosine and A_1 receptors in the cerebellum, cortex, and striatum in the acute ataxic effects of alcohol. Thus, local infusion of A_1 receptor agonists or antagonists in those brain areas increases or decreases, respectively, the motor-impairing effects of alcohol.[55–59] The recent studies by Thakkar and coworkers strongly suggest that alcohol-induced increase in adenosine in the basal forebrain and stimulation of A_1 receptors in the cholinergic neurons of the corticopetal basal forebrain arousal system is particularly involved in the acute somnogenic effects of alcohol.[60–62] First, they showed with *in vivo* microdialysis that local perfusion of alcohol elevates significantly the extracellular concentration of adenosine in the basal forebrain.[60] Second, systemic alcohol administration reduced the activity of cholinergic cells in the basal forebrain and the bilateral microinjection of an A_1 receptor antagonist in the same brain region significantly attenuated alcohol-induced sleep.[61] In relation to anxiety, several studies have shown the involvement of A_1 receptors. A_1 receptor knockout mice display enhanced anxiety,[63] and the anxiogenic effects of caffeine have been generally attributed to blockade of A_1 receptors, although a role of A_{2A} receptors cannot be discarded.[64] Nevertheless, using selective A_1 and A_{2A} receptor ligands, Prediger *et al.* clearly showed that only A_1 agonists and antagonists are able to potentiate and reduce, respectively, the anxiolytic-like effect of alcohol in the elevated plus-maze in mice.[65]

What are the mechanisms of increased adenosine tone after acute administration of alcohol? One mechanism is the

metabolism of alcohol by the liver, which produces acetate that can be metabolized to adenosine in the brain.[66] But the main mechanism seems to be a direct inhibition of the equilibrative nucleoside transporter (ENT1). This effect was first demonstrated *in vitro*, in cells in culture.[67] As expected, mice lacking ENT1 show reduced effects of acute administration of alcohol, such as less somnogenic and ataxic responses.[68] On the other hand, transgenic overexpression of human ENT1 in mouse neurons increases sensitivity to the acute intoxicating effects of alcohol.[69] In summary, the ENT1-dependent increase in the extracellular concentration of adenosine, by acting on A_1 receptors localized in different brain areas, seems to play a very important role in the ataxic, somnogenic, and anxiolytic effects of the acute administration of alcohol.

Does adenosine also play any role in the reinforcing effects of alcohol? In fact, as mentioned before in relation to the interactions between A_{2A} and D_2 receptors in the ventral striatum, an increased adenosine tone should impair dopamine neurotransmission by decreasing the effects dopamine release induced by alcohol. In fact, A_{2A} receptor activation decreases alcohol consumption.[70] Therefore, alcohol-induced increase in extracellular adenosine seems to act as a brake for the reinforcing effects associated with the acute administration of alcohol. It must, however, be mentioned that another line of research suggests that A_{2A} receptor stimulation, under certain conditions, synergizes with D_2 receptor stimulation in the ventral striatum and it has been suggested that this mechanism could be involved in the reinforcing effects of alcohol.[36] According to this rationale, A_{2A} receptor antagonists could be useful in preventing alcohol abuse, and some preclinical data support this possibility.[71] However, another situation has to be taken into account under conditions of chronic alcohol consumption, where adenosine, adenosine receptors, and ENT1 also play a key but different role, compared with the acute situation.

Adenosine Mechanisms in the Chronic Pharmacological Effects of Alcohol

Contrary to the acute situation, in the chronic alcohol situation, we have a reduced adenosine tone. In fact, chronic alcohol exposure results in a decreased expression of ENT1 and, therefore, a decrease in alcohol-mediated inhibition of ENT1.[69] The loss of reuptake of adenosine after chronic exposure to alcohol is most probably the main mechanism by which alcohol tolerance develops both in cell lines[69] and animals.[72] Several studies have shown that the consequent reduction in the adenosine tone is associated with an upregulation of A_1 receptors, which seems to be at least partially responsible for the tolerance to the acute effects of alcohol and also to the main symptoms of alcohol withdrawal, such as insomnia, anxiety, and seizures (Fig. 1).[73–77]

Choi *et al.* showed that alcohol consumption in ENT1 knockout mice was significantly higher than that of wild-type littermates.[68] The pharmacological effects of alcohol in ENT1 knockout mice were in fact associated with a decrease in adenosine tone, demonstrated as a decrease in A_1 receptor–mediated modulation of striatal glutamatergic neurotransmission.[68] Other experimental results also indicate that ENT1 expression is inversely correlated with alcohol consumption. Thus, ENT1 expression is significantly higher

in the alcohol-avoiding CD1 outbred mouse strain than in the alcohol-preferring C57BL/67 inbred strain or in hybrid C57BL/67X CD1 mice, which also displays alcohol-preferring behavior.[78]

The question is then why a reduced adenosine tone is associated with increased alcohol consumption. Apart from its role in tolerance and withdrawal, a decreased adenosine tone should potentiate dopamine neurotransmission in the ventral striatum by means of A_{2A}-D_2 receptor interactions and, therefore, potentiate the effects of alcohol on dopamine release in the ventral striatum. In fact, A_{2A} receptor activation decreases alcohol consumption.[70] In line with the particular involvement of A_{2A} and D_2 receptors, D_2 receptor knockout mice show a marked reduction in alcohol preference,[79] while A_{2A} receptor knockout mice drink more alcohol.[70,80] About the efficacy of A_{2A} receptor antagonists in decreasing alcohol consumption in rats,[71,81] we believe that other mechanisms are involved, such as the blockade of presynaptic A_{2A} receptors in striatal glutamatergic terminals.[82,83] This mechanism has been recently suggested to be involved in the ability of an A_{2A} receptor antagonist to counteract cannabinoid-induced self-administration in monkeys.[84]

Adenosine Mechanisms in the Alcohol-Caffeine Combination

During acute alcohol intake, caffeine largely antagonizes the "unwanted" effects of alcohol by blocking A_1 receptors, which mediate alcohol's somnogenic and ataxic effects. On the other hand, alcohol-induced increase in the extracellular concentration of adenosine can decrease the A_1 receptor–mediated "unwanted" anxiogenic effects of caffeine.[29] The mutual antagonism of "unwanted" effects gives the possibility of increasing significantly the intake of both drugs in the pursuit of the "wanted" reinforcing effects. Further, the striatal A_{2A}-D_2 receptor interactions provide a mechanism by which caffeine can potentiate the reinforcing effects of alcohol, since blockade of ventral striatal A_{2A} receptors can potentiate postsynaptically the alcohol-mediated dopamine release. During chronic alcohol intake, in addition to providing a mechanism for counteracting tolerance to the acute effects, by blocking the effects of upregulated A_1 receptors, caffeine provides a "treatment" for the withdrawal effects of alcohol. Further, blockade of A_{2A} receptors by caffeine most likely contributes to the "wanted" reinforcing effects of alcohol, which probably depend on an already decreased inhibitory tone of adenosine on ventral striatal dopamine neurotransmission.

In summary, adenosine neurotransmission is a unique mechanistic link between caffeine and alcohol, and provides an explanation for the potentially risky effects when the two substances are combined.

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