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Caffeine and the Olfactory Bulb

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Abstract

Caffeine, a popular CNS stimulant, is the most widely used neuroactive drug. Present in coffee, tea, chocolate, and soft drinks as well as over-the-counter and prescription medications, it influences millions of users. This agent has achieved recent notoriety because its dependency consequences and addictive potential have been re-examined and emphasized. Caffeine's central actions are thought to be mediated through adenosine (A) receptors and monoamine neurotransmitters. The present article suggests that the olfactory bulb (OB) may be an important site in the brain that is responsible for caffeine's central actions in several species. This conclusion is based on the extraordinarily robust and selective effects of caffeine on norepinephrine (NE), dopamine (DA), and particularly serotonin (5HT) utilization in the OB of mice. We believe that these phenomena should be given appropriate consideration as a basis for caffeine's central actions, even in primates. Concurrently, we review a rich rodent literature concerned with A, 5HT, NE, and DA receptors in the OB and related structures along with other monoamine parameters. We also review a more limited literature concerned with the primate OB. Finally, we cite the literature that treats the dependency and addictive effects of caffeine in humans, and relate the findings to possible olfactory mechanisms.

Index Entries: Caffeine; olfactory bulb; serotonin; norepinephrine; dopamine; monoamine utilization; stimulation; dependency.

Introduction

Caffeine is the most widely used central nervous system (CNS) stimulant, and lately, its dependency properties have been emphasized (Griffiths et al., 1986; Wise, 1987; Graham, 1988; Benowitz, 1990; Elkind, 1991; Jack, 1991; Chou, 1992; Evans and Griffith, 1992; Kozlowski et al., 1993; Strain et al., 1994). It is believed that both caffeine's excitatory and dependency effects may be mediated by antagonizing adenosine (A) receptors and altering the synthesis, turnover, and concentration of central monoamines (Wise, 1987; Hadfield and Milio, 1989; Kirch et al., 1990; Erfurth and Schmauss, 1990; Daly, 1993; Sawynok and Yaksh, 1993; Fredholm, 1995). However, further work is required to determine the precise neurochemical substrates related to these clinical consequences and where they occur in the brain.

For this reason, we studied the effect of caffeine on the utilization of serotonin (5HT), norepinephrine (NE), and dopamine (DA) in several brain regions of mice (Hadfield and Milio, 1989). Monoamine utilization was most markedly altered in the olfactory bulb (OB), although caffeine also produced noteworthy monoamine changes in the olfactory tubercles (OT), prefrontal cortex (PC), amygdala (AMY), hypothalamus (HT), and thalamus (TH). In still other brain regions, caffeine produced no changes in these monoamine neurotransmitter systems. The predominant effect of caffeine on OB monoamines may help explain why patients who receive caffeine infusions during sleep report olfactory hallucinations (Koenigsberg et al., 1993).

The present article highlights the extraordinarily robust actions of caffeine on monoamine utilization in the OB of mice and, to a lesser extent, other limbic structures. We believe that these phenomena may be primarily responsible for caffeine's prominent CNS stimulatory and dependency effects. The data are discussed in light of pertinent A and monoamine receptor studies and other studies concerned with the effects of caffeine on monoamine systems and behavior as they relate to the OB of rodents and primates.

Methods

Adult white male ICR mice (6 ± 1 wk old, Dominion Laboratories, Dublin, VA) were housed, fed, and tended under carefully controlled conditions as supervised by a doctor of veterinary medicine. All efforts were made to reduce animal suffering and the number of animals used. Groups of 10 animals each received 0, 100, or 200 mg/kg caffeine, ip, 30 min prior to brain removal. High caffeine doses were chosen to produce an unequivocal biochemical effect, although they produce drug levels higher than normally found in humans. The OB, OT, PC, septum (SP), striatum (ST), AMY, HT, hippocampus (HC), and TH were dissected on a chilled glass plate over liquid nitrogen. The tissues were homogenized, and the monoamines were extracted in acetate buffer and then injected into our high-performance liquid chromatography (HPLC) system, as previously described (Hadfield et al., 1986a,b; Hadfield and Milio, 1987a,c). The data were recorded as ng of monoamine/g of brain tissue for: 5HT, NE, DA, 5-hydroxyindoleacetic acid (5HIAA), 3-methoxy-4-hydroxyphenylglycol (MHPG), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 3-methoxytyramine (3MT). The ratios of the metabolite to neurotransmitter levels served as an indicator of neurotransmitter utilization. The data were analyzed using a one-way analysis of variance (ANOVA) followed by Neuman-Keuls post-hoc tests for a determination of the individual differences.

Results

The specific values for each monoamine determination are published elsewhere (Hadfield and Milio, 1989). The data were highly reproducible as shown by the replication of a caffeine effect at the two doses, with the values typically being dose-dependent. The probability values by ANOVA were highly significant ($p < .0005$) and *post-hoc* tests revealed a number of significant monoamine differences for individual brain regions. The results are summarized below, as the degree of greatest change in the metabolite/neurotransmitter ratios produced over drug-free values, regardless of caffeine dose (i.e., the approximate number of times the ratios were increased).

Serotonergic Systems

5HIAA/5HT was increased 10× in the OB and minimally decreased in the HT.

Noradrenergic Systems

MHPG/NE was increased in the OB (×3.5), SP (×2.75), HT (×2.5), OT (×2), PC (×2), AMY (×1.75), and TH (×1.75).

Dopaminergic Systems

DOPAC/DA was increased in the SP (×2.75) and PC (×2). HVA/DA was increased in the SP (×4), HT (×3.25), and OT (×2). 3MT/DA was increased only in the OB (×4). Bar graphs of significant metabolite/neurotransmitter ratio changes in the OB are shown in Fig. 1.

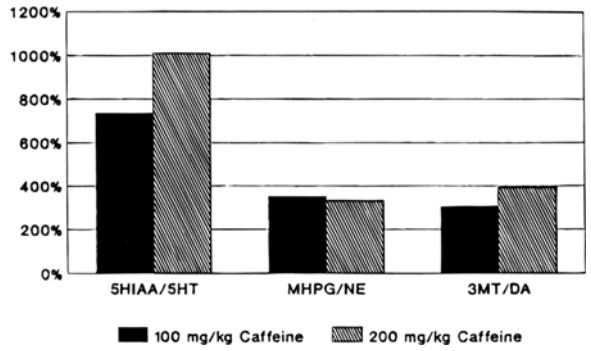

Fig. 1. OB metabolite/transmitter ratios. This is a bar graph representing utilization of 5HT, NE, and DA in the OB of mice as indicated by monoamine metabolite/neurotransmitter ratios. The drug-free control values are set at zero, and the significant caffeine-induced changes in these values, at doses of 100 and 200 mg/kg, are shown as a percent increase over the control values.

Discussion

Why does caffeine exert such a strong preferential effect on monoamine utilization in the OB of mice compared with other important brain regions (Hadfield and Milio, 1989)? In particular, why does caffeine dramatically increase 5HT utilization in the OB, to the exclusion of other brain regions? Does this indicate that the major CNS actions of caffeine—stimulation and dependency—are mediated in large part by OB monoamines? We have performed similar regional brain studies in mice using cocaine (Hadfield and Milio, 1992; Hadfield, 1995), ethanol (Milio and Hadfield, 1992), and rimcazole (BW234U), an experimental neuroleptic agent (Hadfield and Milio, 1987b), but these drugs produced little or no change in monoamine utilization in the OB, in spite of their striking effects on behavior and the limbic system. Therefore, the caffeine effect on OB monoamines appears to be selective. We searched for answers to the above questions by examining the literature for specific differences in A and monoamine receptors in the OB compared with other brain regions.

Caffeine, A Receptors, and the OB

The methylxanthines, including caffeine, are thought to exert their principal effects on neurotransmission by antagonizing A receptors (*see* reviews by Erfurth and Schmauss, 1990; Sawynok and Yaksh, 1993; Fredholm, 1995). This disinhibits transmitter release and would account for the increase in metabolite/neurotransmitter ratios seen in our study. Several radioautography studies indicate that A receptors are relatively dense in the OT (Lee and Reddington, 1986; Fastbom et al., 1987; Jarvis et al., 1989; Jarvis and Williams, 1990), but there is little information available for the OB. However, a radioautograph of the OB illustrated by Jarvis et al. (1989) shows that A2 receptors are well represented in the OB, but less dense than in the OT, and on a par with many other brain regions. Their presence in the OB must be important because precocious development of A deaminase-containing neurons can be seen projecting to the rat OB by embryonic d 15 (Senba et al., 1989).

Caffeine, Serotonergic Mechanisms, and the OB

There are several autoradiographic studies for 5HT1 and 5HT2 receptor subtypes that include the OB (Pazos et al., 1985a,b; Fishette et al., 1987; McKenna and Saavedra, 1987; Hoffman and Mezey, 1989; McKenna et al., 1989; Appel et al., 1990). The density for S1 and S2 receptors in the OB is intermediate to that seen in other brain structures, with S1 > S2 (Fishette et al., 1987). This contrasts with a particularly high density of 5HT receptors in the adjacent OT. In some of these studies, the data for the OB are not expressed in numerical

values, but can be appreciated to some degree by visual inspection of the radioautographic plates. In other studies, 5HT1c receptor mRNA expression in the OT and in the olfactory nuclei of the OB is intermediate to that seen in other brain areas (Hoffman and Mezey, 1989), as is the molecular expression of the 5HT7 serotonin receptor subtype (Shen et al., 1993). Since 5HT receptors have now been reclassified into several subsets (Martin and Humphrey, 1994), further investigations may determine that there is a specific richness in the OB for one or more of these subsets and that some of them may prove particularly sensitive to caffeine. Finally, there are at least two significantly different anatomic types of 5HT axonal systems—one consisting of fine fibers and one consisting of denser, beaded fibers. The latter, which are greater in diameter and more resistant to the toxic effects of amphetamine derivatives, supplies the OB (Mamounas et al., 1991). This may help account for some of the serotonergic differences noted.

5HT is critical to olfactory learning, as demonstrated in neonate rat pups (McLean et al., 1993). Olfactory bulbectomy has also been promoted as a model of agitated hyposerotonergic depression (Lumia et al., 1992). In this regard, many of the alerting actions of caffeine are thought to be related to effects on 5HT neurons (Nehlig et al., 1992), including increased locomotor activity (Reith, 1990). Moreover, caffeine may directly induce the serotonergic "head twitch" response in mice produced by 5HTP, without prior treatment (Kitatani et al., 1993).

Caffeine, Catecholaminergic Mechanisms, and the OB

As with 5HT receptors, the distribution and density of NE and DA receptors in the OB do not appear to be peculiar compared with other limbic brain regions, being essentially intermediate in amount (Daly, 1993). Both NE and DA play an important role in olfaction. Noradrenaline depletion of the OB induces cannibalism at parturition in primiparous mice because olfactory recognition of their pups is impaired (Calamandrei et al., 1992). Self-biting induced by caffeine appears to be mediated by both noradrenergic and dopaminergic mechanisms (Minana and Grisolia, 1986). Dopaminergic systems have been linked primarily to increased locomotion produced by caffeine (Ferré et al., 1991). This caffeine-induced locomotor effect is attenuated by destroying DA neurons with 6-hydroxy-DA (Waldek, 1973; Erinoff and Snodgrass, 1986) or by inhibiting catecholamine synthesis with α-methyltyrosine (White et al., 1978; White and Keller, 1984). DA systems appear to be closely linked to the behavioral effects of A analogs (Daly, 1993), and these may involve the A2a receptors that coexist with DA receptors in the limbic system (Ferré et al., 1991). Caffeine potentiates the behavioral stimulant responses to:

1. Amphetamine, which releases DA (Schechter, 1977; Clans et al., 1974);
2. Cocaine, which blocks reuptake of DA (Misra et al., 1986); and
3. Apomorphine, a directly acting DA agonist (Clans et al., 1974).

Of interest is the selective effect of caffeine on "limbic" vs "extrapyramidal" DA neurons. In in vivo rats, caffeine increases the activity of VTA A10 DA neurons, but not substantia nigra A9 DA neurons (Stoner et al., 1988). This is consonant with the data of the present study, which show a more reactive "limbic" than "extrapyramidal" response to caffeine.

From the above review, it is evident that the density of neither A receptors nor of 5HT, NE, or DA receptors is sufficiently high in the OB to account for the enormous and selective response of 5HT systems, and the greater response of DA and NE systems to caffeine in the OB. Perhaps binding studies with radiolabeled caffeine may reveal a specific caffeine receptor in the OB that would be particularly rich and dense compared with other brain regions. Indeed, Missak (1991) suggests the presence of an endogenous caffeine-like substance in the brain. Alternately, the A receptors themselves may be more highly

sensitive to caffeine in the OB, and caffeine would thus exert a greater effect on the release of 5HT and other monoamines in the OB than in brain structures remote from it. This would depend in part on the mix or blend of high- and low-affinity systems. In addition, we know that caffeine is capable of altering the density of a variety of 5HT, DA, and NE receptors, at least chronically (Shi et al., 1993).

Most of the above data are based on rodent studies. OB work in primates is limited, and the olfactory system is less well developed in them, particularly in humans. Nevertheless, there are many similarities. In lemurs, efferents from the OB extend through the medial forebrain bundle to limbic, basal ganglia, hypothalamic, and brainstem structures, where they make synaptic connections with major aminergic nuclei involved in neurovegetative, neuroendocrine, and behavioral regulation (Mestre et al., 1992). Moreover, Reyher (1988) finds that the connective patterns of the pars externa system of the anterior olfactory nucleus with the main OB parallels the organization in subprimates. When the OB and OT become outlined in the developing human brain, nerve fibers from them are seen to arrive at and pass through the amygdaloid area (Muller and O'Rahilly, 1989).

As in rodents, major neuropeptide transmitters have been identified in the OB of primates, for example, corticotropin-releasing factor (CRF) in monkeys (Bassett et al., 1992) and substance P in marmosets (Sanides-Kohlrausch and Wahle, 1991). For angiotensin, the pattern in the OB of African green monkeys resembles that of gerbils more closely than that of rats (Petersen et al., 1985).

In primates, the behavioral effects of bulbectomy are less marked than in rodents. Contrary to cricetid rodents, a response to photoperiodic signals for sexual activity is not prevented by OB removal in lemurs (Perret and Schilling, 1993) although testosterone levels are altered. The effects of light cycles on sexual activity and reproductive physiology may be mediated by a retinal projection to the OT (Mick et al., 1993). In humans, acquired loss or congenital lack of OBs and olfactory tracts, such as in Kallman syndrome (Wortsman and Hughes, 1996), does not tend to produce behavioral changes, although, of course, anosmia results. Absence of the OB is also common in holoprosencephaly and other malformation syndromes, but its role in olfactory nerve development and the morphogenesis of nasal structures is unknown. According to Braddock et al. (1995), the olfactory receptor cells and olfactory nerves are present in arrhinencephaly, but do not make contact with the brain.

Relationship to Caffeine Dependency

Caffeine is the most widely used and abused drug. Its use may lead to a state of dependency in humans, but caffeine's addictive effects tend to be overlooked since virtually everyone ingests this socially acceptable, legal agent—in coffee, tea, chocolate, soft drinks, over-the-counter medications, and prescription drugs; and the deleterious repercussions are less severe than for "hard" drugs like cocaine and heroin. Withdrawal produces a variety of transient dysphoric symptoms, including headache, fatigue, and irritability, whereas abstinence tends to improve psychological functioning in patients suffering from anxiety, depression, and headaches (Jack, 1991). The dependency potential of caffeine and its consequences have recently been reviewed and re-emphasized by several workers (Griffiths et al., 1986; Wise, 1987; Graham, 1988; Benowitz, 1990; Elkind, 1991; Jack, 1991; Chou, 1992; Evans and Griffith, 1992; Kozlowski et al., 1993; Strain et al., 1994). The study of Strain et al. (1994), from Griffiths laboratory, has received wide acclaim for establishing without reservation that caffeine use is associated with a substance dependence syndrome similar to that seen with other psychoactive drugs. Moreover, these authors conclude that it is important to recognize caffeine dependence as a "clinical syndrome." Although they fall short of labeling caffeine a drug of addiction, their study shows that caffeine meets all four of the well-known major "addiction" criteria.

Graham (1988) points out that addictive drugs tend to activate approach mechanisms ("positive reinforcement"), which are subserved by the medial forebrain bundle and associated structures that induce forward locomotion. The locomotor stimulation and positive reinforcement effects of psychomotor stimulants, like caffeine, result from their activation of these mechanisms. The OB must play a major role in these processes, since this structure mediates the olfactory recognition of such key reinforcing stimuli as food and sex partners, and receives important 5HT, NE, and DA inputs from the brainstem via the medial forebrain bundle. The nerve endings from these fibers synapse with granule cells and other neurons in the OB. Moreover, the OB contains the only well-defined DA cell bodies in the CNS outside the brainstem (the A15 system), where DA has been identified in the periglomerulas (PG), tufted, and short-axon cells (*see* review by Kratskin, 1995). We have not determined precisely how caffeine produces its effects on OB monoamines in mice. Since the alterations are so marked, however, it indicates to us that the OB and related limbic structures must play an important, if not a dominant role in eliciting caffeine's CNS stimulatory and dependency effects (if caffeine produces the same changes in humans). A practical test of this hypothesis would be to assess caffeine dependency in selected patients whose OBs are missing. In conclusion, we hope that these data will serve as a stimulus for further basic and clinical research concerning the actions of caffeine on monoamine systems in the OB, and their relationship to caffeine's CNS stimulatory and dependency effects.

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