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* S-(−)-3-(3-t-Butylamino-2-hydroxypropoxy)-4-morpholino-1,2,5-thiadiazole maleate.

Effects of caffeine on central monoamine neurons

There is strong evidence that cyclic adenosine-3′,5′-monophosphate (cyclic AMP) plays an important regulating role in the function of the nervous system (see book edited by Greengard & Costa, 1970). The data so far support both a presynaptic and a postsynaptic action of cyclic AMP in synaptic transmission (Breekenridge & Bray, 1970; Hoffer, Siggins & Bloom, 1970). In line with this view it has been found that caffeine and theophylline, which are known to inhibit the catabolic enzyme cyclic 3′,5′-nucleotide phosphodiesterase (Butcher & Sutherland, 1962), can release noradrenaline in the mammalian brain (Berkowitz, Tarver & Spector, 1970). On the other hand, it has recently been pointed out that methylxanthines fail to augment cyclic AMP concentrations in brain (Sattin, 1971). Therefore the pharmacological effects of these compounds may not necessarily be mediated via inhibition of the phosphodiesterase (Rall & Sattin, 1970). The present paper confirms the results of Berkowitz & others (1970) and provides evidence for the view that methylxanthines can cause an increase in noradrenaline turnover, but a decrease of 5-hydroxytryptamine (5-HT) and particularly of dopamine turnover.

Male Sprague-Dawley rats (150–200 g) in groups of 4–8 were used. Dopamine, noradrenaline and 5-HT turnover were evaluated by examining the decline of the amine stores after treatment with the tyrosine hydroxylase inhibitor, α-methyl-*p*-tyrosine methylester (H44/68) and the tryptophan hydroxylase inhibitor α-propyldopacetamide (H22/54) (see review by Andén, Corrodi & Fuxe, 1969). Both biochemical and histochemical amine analysis were made (Bertler, Carlsson & Rosengren, 1958; Carlsson & Waldeck, 1958; Carlsson & Lindqvist, 1962; Bertler, 1961; Falck, Hillarp & others, 1962; Corrodi & Jonsson, 1967). Caffeine citrate was given (i.p.) 15 min before the injection of H44/68 (250 mg/kg, i.p.) or of H22/54 (500 mg/kg, i.p.) 4 and 3 h before animals were killed, respectively. All the doses used refer to the base. Histochemically, the effects of aminophylline (theophylline ethylenediamine, 200 mg/kg, i.p.) were studied on the H44/68-induced disappearance of catecholamine fluorescence and on the H22/54-induced disappearance of 5-HT fluorescence. Aminophylline was given 1 h before the inhibitor.

The effects of caffeine administered *in vitro* (10^{-5} or 10^{-4}M) or *in vivo* (100 mg/kg, i.p., 2 h) on the *in vitro* uptake and retention of [^{3}H]noradrenaline (^{3}H-NA) (10^{-7}M) or ^{14}C-5-HT (10^{-6}M) in slices from the neostriatum and the cerebral cortex have also

been investigated. For details of the *in vitro* technique, see Jonsson, Hamberger & others (1969).

Possible interaction of caffeine with amphetamine-induced dopamine release from nerve terminals in neostriatum (see Carlsson, Fuxe & others, 1966) was evaluated in rats with a unilateral chronic lesion of the nigro-neostriatal dopamine pathway (Andén, Dahlström & others, 1966). The extent of the lesion was similar to that described by Hökfelt & Ungerstedt (1968). In this model an increased dopamine release induced, e.g. by amphetamine, will result in rotation of the animal towards the lesioned side, due to a dominance of the intact side. Caffeine (100 mg/kg, i.p.) was given 1 h before amphetamine (1 mg/kg, i.p.).

The effects of caffeine on amphetamine-induced and apomorphine-induced locomotion was studied in an activity box (Animex, Farad, Sweden). Caffeine (100 mg/kg, i.p.) was given 1 h before amphetamine (4 mg/kg, i.p.) or apomorphine (5 mg/kg, i.p.). Apomorphine is a potent dopamine receptor stimulating agent (Ernst, 1967; Andén, Rubenson & others, 1967). Effects of caffeine on the 5-HT mechanisms were evaluated on the extensor hindlimb reflex of acutely spinalized rats which is highly dependent on 5-HT receptor activity (Andén, 1968; Meek, Fuxe & Andén, 1970).

The biochemical results are summarized in Tables 1 and 2. Treatment with caffeine alone did not cause any marked changes in the endogenous monoamine

Table 1. *The effects of caffeine on the extent of dopamine and noradrenaline depletion by H44/68 in whole brain.* Caffeine was given i.p. 15 min before H44/68 (250 mg/kg, i.p., 4 h before killing). n = number of animals. Statistical evaluation by Student's *t*-test.

Treatment	Dose mg/kg	n	Noradrenaline ng/g	Dopamine ng/g
No drug treatment		4	370 ± 14	537 ± 8
Caffeine	100	4	356 ± 16	501 ± 23
Caffeine	50	4	425 ± 17	567 ± 18
Caffeine	25	4	334 ± 13	499 ± 21
H44/68		12	202 ± 5 (e)	156 ± 8 (a)
Caffeine + H44/68	100	4	174 ± 7 (f)	294 ± 16 (b)
Caffeine + H44/68	50	4	190 ± 10	276 ± 23 (c)
Caffeine + H44/68	25	4	193 ± 3	228 ± 20 (d)

(a)–(b): $P < 0.001$; (a)–(c): $P < 0.001$; (a)–(d): $P < 0.001$; (e)–(f): $P < 0.02$.

Table 2. *The effects of caffeine on the H22/54 induced disappearance of 5-HT in whole brain.* Caffeine was given i.p. 15 min before H22/54 (500 mg/kg, i.p., 3 h before killing). n = number of animals. One rat brain was used in each experiment. Statistical evaluation by Student's *t*-test.

Treatment	Dose mg/kg	n	5-HT ng/g
No drug treatment		4	326 ± 31
Caffeine	100	4	355 ± 21
Caffeine	50	4	399 ± 17
Caffeine	25	4	331 ± 37
H22/54		12	152 ± 9 (a)
Caffeine + H22/54	100	4	198 ± 6 (b)
Caffeine + H22/54	50	4	204 ± 6 (c)
Caffeine + H22/54	25	4	148 ± 13

(a)–(b): $P < 0.02$; (a)–(c): $P < 0.02$.

concentrations, although it was observed that caffeine in a dose of 50 mg/kg caused a slight increase in the noradrenaline, dopamine and 5-HT stores. Caffeine in doses of 25 and 50 mg/kg did not produce any significant changes in the extent of noradrenaline depletion brought about by H44/68, whereas a dose of 100 mg/kg resulted in an increase in the amount of noradrenaline that disappeared, suggesting an increase in its turnover after this dose. A dose-dependent decrease in the extent of dopamine depletion caused by H44/68 was observed with caffeine (25–100 mg/kg), suggesting a decrease of dopamine turnover after caffeine. Caffeine (50–100 mg/kg) also decreased the extent of 5-HT depletion brought about by H22/54, suggesting a decrease of 5-HT turnover caused by caffeine. Histochemically, similar results were found. Thus, with a dose of 100 but not 50 mg/kg of caffeine there was an increase in the amount of noradrenaline fluorescence that H44/68 induced to disappear from nerve terminals in both the cortex cerebri and the hypothalamus. There was a marked decrease in the amount of dopamine fluorescence that H44/68 induced to disappear in the dopamine nerve terminals of the neostriatum, the limbic forebrain and the median eminence after doses of caffeine from 50–100 mg/kg. Similarly, with these doses there was a clearcut reduction of the amount of 5-HT fluorescence that H22/54 induced to disappear in the nucleus suprachiasmaticus. Aminophylline (200 mg/kg) induced similar changes in the amounts of noradrenaline, dopamine and 5-HT fluorescence that H44/68 and H22/54 induced to disappear respectively, as did caffeine in a dose of 100 mg/kg when given 1 h before the inhibitor.

We did not observe any significant changes in the *in vitro* uptake and retention of ^{3}H-NA or ^{14}C-5-HT in slices from cerebral cortex or neostriatum after administration of caffeine *in vitro* (10^{-5}M or 10^{-4}M) or *in vivo* (100 mg/kg, i.p., 2 h).

The rotations induced by amphetamine were much reduced by pretreatment with caffeine, 100 mg/kg. Similarly, the amphetamine-, but not the apomorphine-, induced increase in locomotion was reduced (approximately 50%) by pretreatment with caffeine in the same dose as recorded with the activity box. Caffeine alone did not induce rotations or any changes in locomotion. In the extensor hindlimb reflex model, caffeine (25–100 mg/kg, i.p.) was ineffective in changing reflex activity.

The present data confirm and extend the results of Berkowitz & others (1970) indicating that methylxanthines can increase noradrenaline turnover in various parts of the brain. This is also supported by results recently published by Waldeck (1971), who observed in mice that methylxanthines cause an increase in the extent of noradrenaline depletion after inhibition of dopamine β-hydroxylase and an increase in ^{3}H-NA accumulation after injection of [^{3}H]tyrosine.

The present results furthermore indicate that caffeine and aminophylline decrease 5-HT and particularly dopamine turnover as studied in a 3 and 4 h interval, respectively. Consistent with this view Waldeck (1971) found in a 2 h interval a slight decrease in the extent of dopamine depletion induced by H44/68 and a reduction in [^{3}H]dopamine accumulation from [^{3}H]tyrosine in mice after caffeine in a dose of 50 mg/kg. In the early time interval (30 min), however, caffeine was found to increase the accumulation of [^{3}H]dopamine after injection of [^{3}H]tyrosine (Waldeck, 1971), suggesting an increased dopamine turnover at this time interval. Thus, the effects of caffeine on dopamine turnover seems to be biphasic with an initial increase followed by a prolonged decrease. The functional experiments suggest that caffeine is neither a dopamine nor a 5-HT receptor stimulating agent but might possibly act presynaptically to reduce dopamine and 5-HT release from the nerve terminals as indicated by the results obtained from the experiments on locomotion and rotational behaviour. Therefore, it is possible that the observed decrease in dopamine and 5-HT turnover induced by methylxanthines might be due to inhibition of transmitter release although the present studies on the uptake and retention of labelled amines

gave negative results (*cf.* Berkowitz & others, 1970). Another possibility is that the observed effects on monoamine turnover could be due at least in part to changes in nervous impulse flow. However, the findings of an enhanced formation of noradrenaline after monoamine oxidase inhibition by caffeine (Berkowitz & others, 1970) and also of 5-HT (unpublished data) point to a local action of methylxanthines at the level of the axon terminal possibly by interference with the end-product inhibition mechanism. At present it seems difficult to relate this effect to the monoamine turnover changes observed. It is not known whether the effects of methylxanthines on monoamine turnover and synthesis are associated with their ability to block the phosphodiesterase or not.

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