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Some effects of caffeine and aminophylline on the turnover of catecholamines in the brain

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The effect of caffeine and aminophylline on the turnover of noradrenaline and dopamine in the mouse brain was studied by two different methods: estimation of the rate of disappearance of the transmitter after inhibition of synthesis and measurement of the accumulation of [^{3}H]noradrenaline (^{3}H-NA) and [^{3}H]dopamine (^{3}H-DA) after administration of [^{3}H]tyrosine. After inhibition of the tyrosine hydroxylase the xanthines had little or no effect on the disappearance of both catecholamines. After inhibition of dopamine β-hydroxylase, both xanthines increased the rate of disappearance of noradrenaline. When given in sufficiently large doses both xanthines increased the yield of ^{3}H-NA and ^{3}H-DA from [^{3}H]tyrosine. Pretreatment with a monoamine oxidase inhibitor caused a decrease in the yield of the tritiated catecholamines, which could be counteracted by the xanthines. Stimulation of the noradrenaline receptors by clonidine appeared to cause a decrease in the yield of ^{3}H-NA and an increase in the amount of ^{3}H-DA formed from [^{3}H]tyrosine. Conversely, stimulation of the dopamine receptors by apomorphine caused a decrease in the yield of ^{3}H-DA and an increase in that of ^{3}H-NA. Also, in caffeine-treated animals, clonidine and apomorphine decreased the yield of ^{3}H-NA and ^{3}H-DA respectively. However clonidine could not increase ^{3}H-DA concentrations, nor apomorphine the ^{3}H-NA concentrations more than did caffeine alone. Thus, caffeine and aminophylline appear to increase the rate of turnover of both catecholamines in the brain.

Methylxanthines and especially caffeine are ingested daily by most people in coffee, tea and chocolate. As these compounds exhibit a wide range of pharmacological activities (see Goodman & Gilman, 1970) their possible interaction with drugs cannot be ignored. To predict and reveal such interactions, a detailed knowledge of the mode of action of the methylxanthines is essential.

Recently theophylline and caffeine were shown to increase the rate of synthesis of noradrenaline in the brain, as revealed by the use of labelled tyrosine, and to increase the rate of its disappearance after tyrosine hydroxylase inhibition. The two methylxanthines were also able to potentiate the accumulation of noradrenaline in the brain after monoamine oxidase (MAO) inhibition (Berkowitz, Tarver & Spector, 1970).

The present investigation confirms and extends these observations. In addition to a tyrosine hydroxylase inhibitor, a dopamine β-hydroxylase inhibitor, FLA-63 (Carlsson, Corrodi & others, 1970; Florvall & Corrodi, 1970), was used. Moreover, brain dopamine concentrations were also recorded. In an attempt to analyse the action of caffeine on the turnover of catecholamines, clonidine and apomorphine were used to stimulate chemically the noradrenaline and dopamine receptors respectively (Andén, Rubenson & others, 1967; Andén, Corrodi & others, 1970).

METHODS

Female mice, about 20 g were used. Noradrenaline and dopamine were determined spectrophotofluorimetrically, after tissue extraction in perchloric acid and subsequent strong cation exchange chromatography on Dowex 50 columns (Bertler, Carlsson & Rosengren, 1958; Carlsson & Waldeck, 1958; Carlsson & Lindqvist, 1962). [^{3}H]Noradrenaline (^{3}H-NA) and [^{3}H]dopamine (^{3}H-DA) were determined by liquid scintillation counting, after separation on alumina and Dowex columns (Persson & Waldeck, 1968).

Drugs used were: Apomorphine chloride, caffeine, clonidine, α-methyltyrosine methylester (H 44/68), bis-(4-methyl-1-homopiperazinylthiocarbonyl)-disulphide (FLA-63), nialamide and aminophylline. L-Tyrosine-ring-[3,5-^{3}H] was obtained from NEN Chemicals, Dreieichenhein, and from The Radiochemical Centre, Amersham.

The data shown in Table 3 are derived from two experimental series using [^{3}H]-tyrosine from different sources. This caused differences in the yield of [^{3}H]catecholamines which could be compensated for by using the pxq factorial test of Winer (1962).

RESULTS

Effect of caffeine and aminophylline on the disappearance of brain catecholamines after inhibition of their synthesis

Caffeine or aminophylline (50 mg/kg) were given intraperitoneally alone, or together with either of the synthesis inhibitors H 44/68 (200 mg/kg), or FLA-63 (40 mg/kg). Animals receiving either of the synthesis inhibitors alone were run in parallel. Untreated animals served as controls. Two h after the administration of the drugs the animals were killed and the amount of noradrenaline in the brain was determined.

Caffeine had no effect on the concentration of brain noradrenaline (Table 1), whereas aminophylline appeared to cause a slight increase ($P < 0{\cdot}05$). Neither of the two xanthines significantly changed the extent of noradrenaline depletion brought about by H 44/68. After FLA-63, however, noradrenaline disappeared much faster both when caffeine and when aminophylline were given with the inhibitor ($P < 0{\cdot}05$ and 0·025 respectively).

Table 1. *Effects of caffeine and aminophylline on the disappearance of noradrenaline from the mouse brain after inhibition of its synthesis.* Caffeine (50 mg/kg), aminophylline (50 mg/kg), the tyrosine hydroxylase inhibitor H 44/68 (200 mg/kg) and the dopamine β-hydroxylase inhibitor FLA-63 (40 mg/kg) were given intraperitoneally to mice in the combinations shown below. Two h later the animals were killed and the amount of noradrenaline in the brain was determined. Untreated animals served as controls. Shown are the means ± s.e. as in per cent of the control value. Figures in parentheses denote the number of experimental groups, each comprising six animals.

	Synthesis inhibitor		
Drug	None	H 44/68	FLA-63
None	100 ± 4 (5)	62 ± 5 (7)	45 ± 3 (5)
Caffeine ..	98 ± 11 (2)	69 ± 4 (4)	27 ± 1 (2)
Aminophylline	114 ± 2 (4)	67 ± 3 (4)	29 ± 2 (4)

Table 2. *Effects of caffeine and aminophylline on the disappearance of noradrenaline and dopamine from the mouse brain following tyrosine hydroxylase inhibition by H* 44/68. Mice received H 44/68 (200 mg/kg, i.p.) alone, or followed 1 h later by caffeine (50 mg/kg, i.p.) or aminophylline (50 mg/kg, i.p.). Three h after the administration of H 44/68 the animals were killed and catecholamine contents of the brain were determined. Untreated animals served as controls. Shown are the means ± s.e. in per cent of the control value. Figures in parentheses denote the number of experimental groups each comprising six animals.

Control	H 44/68	H 44/68 + caffeine	H 44/68 + aminophylline
	Noradrenaline		
100 ± 1 (4)	65 ± 4 (4)	62 ± 2 (4)	50 ± 3 (3)
	Dopamine		
100 ± 3 (4)	37 ± 1 (4)	45 ± 5 (4)	43 ± 9 (4)

In the next experiment H 44/68 was given 1 h before the xanthines, the animals being killed 3 h after the administration of the inhibitor. Other experimental conditions were the same as above. The concentrations of both catecholamines in the brain were determined (Table 2). In this case the disappearance of noradrenaline after H 44/68 was accelerated by aminophylline ($P < 0{\cdot}005$), whereas caffeine had no effect. The disappearance of dopamine after H 44/68 appeared to be diminished by the xan thines, but this could not be verified statistically due to a large scatter in the groups with the combined treatment.

Effect of caffeine and aminophylline on the yield of ^{3}*H-NA and* ^{3}*H-DA from* [^{3}*H*]*tyrosine*

[^{3}H]Tyrosine (5 μg/kg) was given intravenously to mice. Some animals were given caffeine (50 or 100 mg/kg, i.p.) 30 min or 2 h beforehand, whilst others received aminophylline in the same doses and at the same time intervals. One h after the administration of [^{3}H]tyrosine the animals were killed and their brains removed and analysed for ^{3}H-NA and ^{3}H-DA.

The results in Table 3 show that the lower dose of caffeine did not change the amount of ^{3}H-NA in the brain at any time interval, whereas when given 2 h before [^{2}H]tyrosine it significantly reduced that of ^{3}H-DA ($P < 0{\cdot}05$). When 100 mg/kg of caffeine was given 30 min before [^{3}H]tyrosine, the yield of both [^{3}H]catecholamines was increased twofold ($P < 0{\cdot}001$). When given 2 h beforehand there was no effect. Aminophylline (50 mg/kg) when given 30 min but not 2 h before [^{3}H]tyrosine increased the amount of ^{3}H-NA ($P < 0{\cdot}01$). At a dose of 100 mg/kg of the drug, the amount of both ^{3}H-NA and ^{3}H-DA in the brain was significantly increased when given 30 min before ($P < 0{\cdot}001$ and 0·01 respectively), less significantly (^{3}H-NA, $P < 0{\cdot}05$) or not at all (^{3}H-DA) when given 2 h before [^{3}H]tyrosine.

Effect of caffeine and aminophylline on the yield of ^{3}*H-NA and* ^{3}*H-DA from* [^{3}*H*]*tyrosine after MAO inhibition*

Mice received the MAO inhibitor, nialamide (100 mg/kg, i.p.) 17 h and (50 mg/kg, i.p.) 1 h before the administration of [^{3}H]tyrosine (5 μg/kg, i.v.). Some animals received caffeine (50 or 100 mg/kg, i.p.) together with the second injection of nialamide,

Table 3. *Effects of caffeine and aminophylline on the synthesis of [^{3}H]noradrenaline (^{3}H-NA) and [^{3}H]dopamine (^{3}H-DA) from [^{3}H]tyrosine in the mouse brain.* Mice were given caffeine or aminophylline intraperitoneally 30 min or 2 h before the administration of [^{3}H]tyrosine (5 μg/kg, i.v.). Control animals received [^{3}H]tyrosine alone. One h after the labelled precursor had been given the animals were killed and ^{3}H-NA and ^{3}H-DA in the brain determined. Shown are the means in fmol/g tissue (1 femtomol = 10^{-15} mol). Each mean is based on 4–6 experimental groups. There were six animals per group.

[^{3}H]amine	Control	Caffeine 50 mg/kg 30 min	Caffeine 50 mg/kg 2 h	Caffeine 100 mg/kg 30 min	Caffeine 100 mg/kg 2 h	Aminophylline 50 mg/kg 30 min	Aminophylline 50 mg/kg 2 h	Aminophylline 100 mg/kg 30 min	Aminophylline 100 mg/kg 2 h
^{3}H-NA	15·2	16·9	13·3	31·1‡	16·2	23·9†	15·8	28·2‡	22·8*
^{3}H-DA	42·5	44·9	29·5*	84·0‡	38·7	54·2	39·8	61·7†	48·1

Significantly different from the control: *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$; pxq factorial test, see methods.

whilst others received aminophylline in the same doses. One h after the labelled precursor had been given the animals were killed and their brains analysed for ^{3}H-NA and ^{3}H-DA. Mice receiving [^{3}H]tyrosine alone served as controls.

Pretreatment with nialamide reduced the yield of both ^{3}H-NA and ^{3}H-DA by about 40% ($P < 0.01$ and 0·001 respectively, see Fig. 1). Given in the high dose, caffeine and aminophylline restored the yield of ^{3}H-NA in nialamide-treated mice to the control value ($P < 0.01$) but only aminophylline was able to do the same with the yield of ^{3}H-DA ($P < 0.001$). Other combinations gave intermediate values or no effect at all.

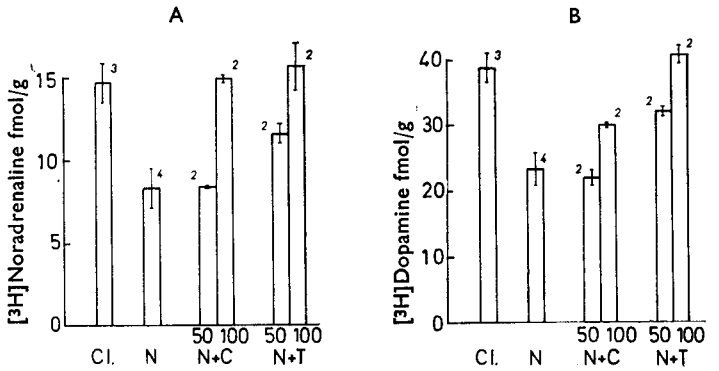

FIG. 1. Effect of caffeine and aminophylline on the synthesis of (A) [^{3}H]noradrenaline (^{3}H-NA) and (B) [^{3}H]dopamine (^{3}H-DA) from [^{3}H]tyrosine in the mouse brain after monoamine oxidase inhibition. Mice were given nialamide (100 mg/kg, i.p.) 17 h and (50 mg/kg, i.p.) 1 h before [^{3}H]tyrosine (5 μg/kg, i.v.). Caffeine or aminophylline was given together with the second injection of nialamide. Controls received [^{3}H]tyrosine alone. The graph shows the amount of ^{3}H-NA and ^{3}H-DA found in the brain 1 h after the labelled precursor had been given. Figures above bars indicate the number of experimental groups (each comprising six animals) from which the mean ± s.e. has been calculated. Cl = control; N = nialamide; C = caffeine, 50 and 100 mg/kg respectively; T = aminophylline, 50 and 100 mg/kg respectively.

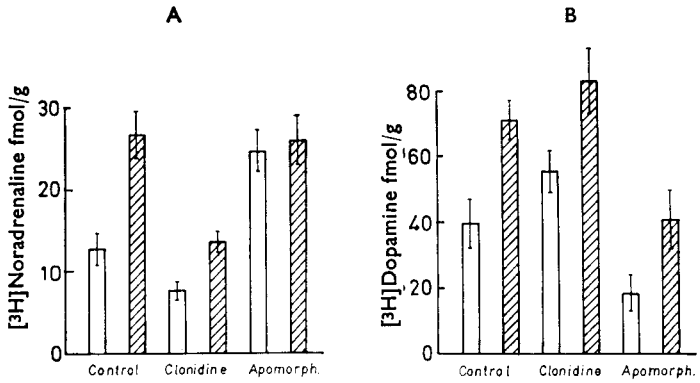

FIG. 2. Effect of caffeine on the synthesis of (A) [^{3}H]noradrenaline and (B) [^{3}H]dopamine from [^{3}H]tyrosine in the mouse brain after chemical stimulation of the receptors. Clonidine (3 mg/kg, i.p.) or apomorphine (25 mg/kg, i.p.) was given alone or together with caffeine (100 mg/kg, i.p.), 30 min before the administration of [^{3}H]tyrosine (5 μg/kg, i.v.). Control animals received [^{3}H]tyrosine alone or in combination with caffeine. The graph shows the amount of [^{3}H]amines found in the brain 30 min after the labelled precursor had been given. The data are the means $\pm$ s.e. of 4–5 experimental groups, each comprising six mice. Shaded columns: caffeine pretreatment.

Effect of caffeine on the yield of ^{3}H-NA and ^{3}H-DA from [^{3}H]tyrosine after chemical stimulation of the catecholamine receptors

Some mice received clonidine (3 mg/kg, i.p.) or apomorphine (25 mg/kg, i.p.) alone, or together with caffeine (100 mg/kg, i.p.). Other animals received caffeine alone. Thirty min later [^{3}H]tyrosine (5 μg/kg, i.v.) was given and after another 30 min the animals were killed and the yield of ^{3}H-NA and ^{3}H-DA in the brain was determined. Controls were given [^{3}H]tyrosine only.

In this experiment also, ^{3}H-NA and ^{3}H-DA in the brain increased after caffeine pretreatment ($P < 0{\cdot}001$ and $0{\cdot}05$ respectively, Fig. 2). After clonidine, ^{3}H-NA tended to decrease whereas ^{3}H-DA if anything increased. However, these changes were not statistically significant. Apomorphine, on the other hand, increased the yield of ^{3}H-NA twofold ($P < 0{\cdot}01$) and reduced the yield of ^{3}H-DA to half the control value ($P < 0{\cdot}001$). The increase in the yield of ^{3}H-NA from [^{3}H]tyrosine brought about by caffeine was antagonized by clonidine ($P < 0{\cdot}01$) but not by apomorphine. In contrast, the increase in ^{3}H-DA brought about by caffeine was antagonized by apomorphine ($P < 0{\cdot}05$) but not by clonidine. In no case was the yield of ^{3}H-NA or ^{3}H-DA higher than after caffeine alone.

DISCUSSION

The measurement of the rate of disappearance of catecholamines after synthesis inhibition is a widely used method of estimating their turnover. The amount of labelled amines accumulating during a short time interval after the administration of labelled tyrosine is another method. Since none of them is quite conclusive (Nybäck & Sedvall, 1970; Persson & Waldeck, 1970a; Wurtman, Anton-Tay & Anton, 1969) both have been used in the present study.

The effect of methylxanthines on the disappearance of brain catecholamines after tyrosine hydroxylase inhibition was slight. Only aminophylline, when given 1 h after H 44/68, increased the rate of disappearance of noradrenaline significantly. The rate of disappearance of dopamine after H 44/68, if anything, decreased after the admini-

stration of the xanthines. When, on the other hand, dopamine β-hydroxylase was inhibited by FLA-63 both xanthines significantly accelerated the disappearance of noradrenaline from the mouse brain. Such a difference in results obtained with the two inhibitors has been observed previously (Persson & Waldeck, 1971) and is thought to be due to an interaction between noradrenaline-containing and dopamine-containing neurons in the brain (Persson & Waldeck, 1970b).

The effects of caffeine and aminophylline on brain noradrenaline after tyrosine hydroxylase inhibition observed by Berkowitz & others (1970) were more pronounced than those reported here. This may in part be due to species differences (rat and guinea-pig as opposed to mouse). However, other causative factors will be discussed below. The slight elevation of the endogenous noradrenaline concentrations observed after aminophylline (Table 1) was probably transient, since it has not been observed by others (cf. Muschol, Kiefer & Lindmar, 1969; Berkowitz & others, 1970).

The experiments on the accumulation of ^{3}H-NA and ^{3}H-DA after [^{3}H]tyrosine indicate an increased turnover of these amines brought about by the xanthines (Table 3). The data also show that 100 mg/kg is a more effective dose than 50 mg/kg and that the effect is of a relatively short duration. This may explain the poor effect noted in the experiments with H 44/68.

When 50 mg/kg caffeine was given 2 h before [^{3}H]tyrosine, the yield of ^{3}H-DA did not increase but rather decreased below the control value. This may indicate a rebound effect which in turn could explain the decreased rate of disappearance of dopamine caused by caffeine in H 44/68-treated animals (Table 2).

Inhibition of MAO results in increased levels of the catecholamines in the brain followed by a reduced turnover (Carlsson, Lindqvist & Magnusson, 1960), probably due to a negative feed-back mechanism for the regulation of catecholamines (Neff & Costa, 1966; Spector & others, 1967). The present experiments, with [^{3}H]tyrosine show that aminophylline, and to a lesser extent also caffeine, were able to increase the yield of ^{3}H-NA and ^{3}H-DA in the brain of nialamide-pretreated animals. This is consistent with the observation that caffeine and theophylline given to animals pretreated with a MAO inhibitor, will raise the endogenous level of brain noradrenaline more than does the MAO inhibitor alone (Berkowitz & others, 1970).

Stimulation of the noradrenaline receptors by clonidine and of the dopamine receptors by apomorphine causes a reduced turnover of the respective transmitters. Here also, an activation of a negative feed-back mechanism has been proposed (Andén & others, 1967; 1970). Conversely, clonidine appears to increase the turnover of dopamine whereas apomorphine increases the turnover of noradrenaline, possibly due to an interaction between these neuronal systems (Persson & Waldeck, 1970b; Persson, 1970). The present data are compatible with these views.

In animals treated with caffeine, clonidine and apomorphine appeared to reduce the turnover of noradrenaline and dopamine respectively to normal values. On the other hand, apomorphine did not increase the noradrenaline, nor clonidine the dopamine, turnover more than did caffeine alone. The interpretation of this interaction is difficult. The results do not clearly indicate an interference by caffeine with the negative feed-back mechanism for the regulation of catecholamine synthesis.

In conclusion, the experiments with synthesis inhibition and with ^{3}H-NA accumulation indicate an increased turnover of noradrenaline in the brain caused by caffeine and aminophylline when given in sufficiently large doses. Also, the turnover of dopamine appeared to be increased by the xanthines as judged from the accumulation

of ^{3}H-DA. How is this increase brought about? Preliminary data show that caffeine is also able to increase the yield of [^{3}H]catecholamines formed from [^{3}H]dopa (unpublished observations), thus suggesting that the site of action is not on the hydroxylation of tyrosine.

Among the biochemical effects of methylxanthines, their inhibitory action on phosphodiesterase, the enzyme responsible for the catabolism of cyclic AMP (Butcher & Sutherland, 1962), has received particular attention. Cyclic AMP appears to act as a second messenger in several regulatory systems (Sutherland & Rall, 1960; Robinson, Butcher & others, 1968) and seems to be involved in transmitter release mechanisms (see Rasmussen, 1970). Consequently, intraneuronal cyclic AMP may be linked to the regulation of catecholamine turnover, in which case the effect of caffeine and aminophylline on the catecholamines described here would be due to inhibition of the catabolism of cyclic AMP. However, more research is necessary to clarify the situation.

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