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LETTERS TO THE EDITOR

Increased dopamine concentration in the striatum in the mouse by FLA-63, a dopamine-β-hydroxylase inhibitor

Injection of the dopamine β-hydroxylase inhibitor bis-(4-methyl-1-homopiperazinyl-thiocarbonyl)disulphide (FLA-63) (Carlsson, Corrodi & others, 1967) reduces the brain noradrenaline concentration in mice and rats (Svensson & Waldeck, 1969; Florvall & Corrodi, 1970; Corrodi, Fuxe & others, 1970a), while the dopamine concentration seems to be unaffected (Svensson & Waldeck, 1969; Corrodi & others, 1970 a), although rather few data have been presented. However, an increase in [^{3}H]dopamine formation from [^{3}H]tyrosine in the brain has been observed initially after FLA-63 administration (Svensson & Waldeck, 1969). This finding has been confirmed by Nybäck (1971), who also found an increase in [^{3}H]dopamine formation from [^{3}H]tyrosine in the striatum. Since this brain region contains very few noradrenaline nerve terminals, but is rich in dopamine nerve terminals, this finding suggests that FLA-63 has an effect on brain dopamine neurons (Nybäck, 1971). I have established a dose-response curve for the effect of FLA-63 on brain concentrations of noradrenaline and dopamine in the mouse and I have studied the effect of FLA-63 on the level of dopamine in the striatum.

Mice were injected intraperitoneally with various doses of FLA-63 and killed 2 h later by decapitation. Amine analyses were made on the pooled brains of 6 mice. The results are shown in Table 1. In other experiments FLA-63, 50 mg kg^{-1}, was given intraperitoneally to mice, which were killed 2½ h later. The striata were dissected from the remainder of the brains and the monoamine concentrations were measured in the respective pooled tissues of 6 mice. The result is shown in Table 2. Noradrenaline was determined according to Bertler, Carlsson & Rosengren (1958) and dopamine according to Carlsson & Waldeck (1958) as modified by Carlsson & Lindqvist (1962). For statistical evaluation, a simple *t*-test, or analyses of variance (with one criterion of classification) followed by *t*-test was used.

FLA-63 at 5 mg kg^{-1} caused a significant reduction in the central noradrenaline concentration (Table 1); but this was not significantly different ($P>0.05$) from that found after a 20 mg kg^{-1} dose. After 40 mg kg^{-1} of FLA-63, the concentration of noradrenaline was reduced compared with that after 20 mg kg^{-1} ($P < 0.001$) and after 80 mg kg^{-1} it was significantly lower than after 40 mg kg^{-1} $P<0.001$) The lower doses of FLA-63 (5–20 mg kg^{-1}) did not significantly affect the dopamine concentration (Table 1), whereas the higher doses, 40 and 80 mg kg^{-1}, significantly increased the

Table 1. *Effect of different doses of FLA-63 on the brain concentrations of noradrenaline and dopamine in the mouse.* Each value is the mean ± s.e. of (n) determinations in μg g^{-1} tissue. FLA-63 was given 2 h before decapitation. * = $P < 0.025$, ** = $P < 0.005$, *** = $P < 0.001$ relative to controls.

	Dose of FLA-63 (mg kg^{-1})					
	0	5	10	20	40	80
		***	***	***	***	***
Noradrenaline	0·40 ± (2)	0·30 ± (3)	0·29 ± (3)	0·28 ± (3)	0·20 ± (3)	0·12 ± (3)
	0·001	0·012	0·011	0·003	0·014	0·003
Dopamine	0·82 ± (3)	0·90 ± (3)	0·94 ± (3)	0·90 ± (3)	1·04** ± (3)	1·00* ± (3)
	0·037	0·056	0·036	0·065	0·065	0·030

Table 2. *Effect of FLA-63, 50 mg kg^{-1}, on the levels of noradrenaline and dopamine in the striatum (dopamine only) and the rest of the brain (noradrenaline and dopamine) in the mouse.* FLA-63 was given 2½ h before decapitation. Shown are the mean of 5 determinations ± s.e. in μg g^{-1} tissue. Controls received isotonic saline (corresponding volume). * = $P < 0.05$, *** = $P < 0.005$.

Treatment	Striatum	Remainder of the brain	
	Dopamine	Noradrenaline	Dopamine
Control	8·22 ± 0·58	0·46 ± 0·012	0·34 ± 0·015
FLA-63	9·95* ± 0·29	0·20*** ± 0·003	0·49*** ± 0·030

dopamine concentration ($P<0.005$ and <0.025, respectively). A significant increase in the dopamine concentration in the striatum ($P<0.05$) was also obtained (Table 2). In the remainder of the brain there was a reduced concentration of noradrenaline ($P<0.001$) and an increased dopamine concentration ($P<0.005$).

Thus it may be concluded that FLA-63, apart from reducing the central noradrenaline concentration, in all probability due to inhibition of dopamine β-hydroxylase, also affects the central dopamine neurons. This view is supported by previous findings (Nybäck, 1971). However, the precise mechanism of action remains unclear. That the three lower doses of FLA-63 seem to cause virtually the same reduction in noradrenaline whereas the two higher doses caused a more pronounced reduction, might indicate that at higher doses of FLA-63 a mechanism of action other than dopamine β-hydroxylase inhibition is involved. This view is supported by previous findings, since judging from data on the effect of FLA-63 on the formation of [^{3}H] dopamine from [^{3}H]tyrosine (Svensson & Waldeck, 1969), the inhibition of dopamine β-hydroxylase is virtually complete at 40 mg kg^{-1}. In spite of this, the noradrenaline concentration was further reduced after FLA-63, 80 mg kg^{-1}, in the present study. It should also be noted that at these, higher doses, the dopamine concentration was significantly increased. It has previously been observed that the disappearance of brain noradrenaline after FLA-63 treatment is more rapid than that after a tyrosine hydroxylase inhibitor (Persson & Waldeck, 1970 a,b; Thierry, Blanc & Glowinski, 1971). Treatment with the dopamine receptor stimulating agent apomorphine accelerates the disappearance of noradrenaline after tyrosine hydroxylase inhibition to the same rate as after FLA-63 alone (Persson & Waldeck, 1970 a,b). This was interpreted as an interaction between central noradrenaline and dopamine neurons. Thus an increased dopamine receptor activity should result in increased activity of the noradrenaline neurons, thereby causing a more rapid depletion of noradrenaline by FLA-63 (cf. also Corrodi & others, 1970 a; Andén & Fuxe, 1971). Hence it is possible that after higher doses of FLA-63 the increased dopamine concentration in the striatum results in increased activation of the dopamine receptors, which then activate the noradrenaline neurons causing the more pronounced depletion of noradrenaline. This view is supported by functional data showing development of sequences of stereotyped behaviour following FLA-63-treatment (Ahlenius & Engel, 1972), since stereotyped behaviour is often associated with dopamine receptor activation (cf. Randrup & Munkvad, 1970). It is also interesting that FLA-63 treatment has been found to enhance the stereotyped behaviour induced by (+)-amphetamine (Corrodi & others, 1970 b) and L-dopa (Ahlenius & Engel, 1971). One might speculate, that the increased dopamine concentration in the striatum is secondary to the noradrenaline depletion in noradrenaline neurons. For example, a reduction in noradrenaline receptor activation probably brought about by FLA-63 might in some way reduce the activity of the dopamine neurons. In this case less dopamine would be released and

hence its concentration might increase. However, injection of the noradrenaline receptor stimulating agent clonidine causes a slight increase in dopamine concentration in the brain in FLA-63 treated animals (Persson & Waldeck, 1970 b), thus arguing against this theory.

It does not seem likely that FLA-63 inhibits monoamine oxidase, in view of the pronounced depletion of noradrenaline obtained. Moreover, treatment with a monoamine oxidase inhibitor, nialamide, antagonizes the FLA-63 induced depletion of noradrenaline (Svensson & Waldeck, 1970). Needless to say, the effect of FLA-63 on central dopamine neurons requires further investigation.

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