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# A new dopamine-β-hydroxylase inhibitor: effects on the noradrenaline concentration and on the action of L-DOPA in the spinal cord

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## Summary

1. The dopamine-β-hydroxylase inhibitor bis(4-methyl-1-homopiperazinyl-thiocarbonyl) disulphide (FLA-63 ; 25 mg/kg i.p.) caused within 4 h a 65% loss of noradrenaline throughout the intact rat spinal cord and also cranial to a transection of the cut spinal cord. Caudal to the lesion, there was only an insignificant depletion of 17% indicating the importance of nerve impulses for the disappearance of noradrenaline.

2. Dopamine accumulated in the spinal cord after treatment with FLA-63 although the amounts were not sufficient to replace the missing noradrenaline. Even after treatment with L-3,4-dihydroxyphenylalanine (L-DOPA), the catecholamine store was incompletely replenished by dopamine.

3. After a large depletion of the noradrenaline stores, induced by repeated doses of FLA-63 or by reserpine plus FLA-63, the L-DOPA-induced increase in flexor reflex activity of the hind limbs of spinal rats was inhibited much more than after pretreatment with α-methyl-tyrosine or reserpine. FLA-63 blocked the formation of noradrenaline but not of dopamine from L-DOPA.

4. The increase in flexor reflex activity induced by the noradrenaline receptor stimulating agent clonidine was not changed by FLA-63, indicating that the noradrenaline receptor sensitivity was not influenced.

5. After depletion of the noradrenaline stores, the small formation of noradrenaline from L-DOPA may be of greater functional significance for the noradrenaline receptor stimulation than the greater formation of dopamine, but the dopamine formed also has a slight action. With intact noradrenaline stores, displacement of endogenous noradrenaline by newly formed dopamine contributes, at least after monoamine oxidase inhibition, to the increase in the flexor reflex activity caused by L-DOPA.

## Introduction

In studies of central catecholamine mechanisms, it is of great interest to differentiate between the roles of dopamine (DA) and noradrenaline (NA). Inhibition of the enzyme DA-β-hydroxylase should be of value in such investigations. This enzyme can be inhibited by various chelating agents (Goldstein, 1966). Thus, disulfiram and its reduced form diethyldithiocarbamate reduce NA but not DA concentrations in the brain (Musacchio, Goldstein, Anagnoste, Poch & Kopin, 1966;

Carlsson, Lindqvist, Fuxe & Hökfelt, 1966). Recently, a structurally related compound, bis(4-methyl-1-homopiperazinylthiocarbonyl) disulphide (FLA-63), was introduced (Carlsson, Corrodi, Florvall, Ross & Sjöberg, 1970; Austrian Patent (1970) No. 284143) which inhibits DA-β-hydroxylase activity and lowers brain NA (Svensson & Waldeck, 1969; Persson & Waldeck, 1970a & b; Florvall & Corrodi, 1970; Corrodi, Fuxe, Hamberger & Ljungdahl, 1970).

The spinal cord offers special advantages in studies on central NA mechanisms since the only neurones containing catecholamines to this region are the bulbospinal NA neurones (Carlsson, Falck, Fuxe & Hillarp, 1964; Andén, Häggendal, Magnusson & Rosengren, 1964; Dahlström & Fuxe, 1965; Andén, Carlsson & Häggendal, 1969). The catecholamine receptors in the spinal cord can, thus, be considered to be of the NA type. Furthermore, a midthoracic cord transection eliminates the nerve impulse flow to the NA terminals in the caudal part of the spinal cord. In this investigation, two particular aspects of the action of FLA-63 have been elucidated in spinalized rats: (1) the importance of the nerve impulses for the disappearance of NA after inhibition of DA-β-hydroxylase, and (2) the role of NA formation for NA receptor stimulation after treatment with the catecholamine precursor L-3,4-dihydroxyphenylalanine (L-DOPA).

## Methods

Male Sprague-Dawley rats weighing 150–220 g were used. Spinal cord transection was performed in the midthoracic region (spinalization) during diethylether anaesthesia. Since this spinalization and several of the drugs used interfered with temperature regulation, care was taken to keep the body temperature normal by changing the environmental conditions. The urinary bladders of the spinal rats were emptied by gentle pressure on the abdomen. The animals had access to food and water.

The following drugs were used: bis(4-methyl-1-homopiperazinylthiocarbonyl) disulphide (FLA-63; * Astra, Södertälje), DL-α-methyltyrosine methylester HCl (H 44/68; * Hässle, Göteborg), reserpine (* Ciba, Stockholm), nialamide (* Pfizer, Näsbypark), L-3,4-dihydroxyphenylalanine (L-DOPA; Ajinomoto, Tokyo), clonidine HCl (Catapresan, St. 155; * Boehringer Ingelheim, Stockholm). The drugs were dissolved in N HCl or in a few drops of glacial acetic acid, when necessary. The final volumes were made up with 5·5% glucose or 0·9% saline solution.

### *Effect of FLA-63 on the concentrations of NA and DA in the intact and transected spinal cord*

For the number of experiments and treatments, see Tables 1 and 2. About half of the animals were spinalized either approximately 6 h before death or the previous evening. FLA-63 (25 mg/kg) was injected intraperitoneally into half of the operated and half of the intact rats 4 h before death. In some histochemical experiments, FLA-63 (20 mg/kg) was given in two doses intraperitoneally 5 and 2 h before death. The rats were killed by exsanguination or decapitation under light chloroform anaesthesia. The cranial and caudal halves of the spinal cord were excised as soon as possible. In the biochemical experiments, the halves from two or three rats were pooled. The NA and DA contents were determined spectrofluorimetrically after cation exchange chromatography and oxidation (Bertler, Carlsson & Rosen-

gren, 1958; Häggendal, 1963; Carlsson & Waldeck, 1958; Carlsson & Lindqvist, 1962). In the histochemical experiments, the fluorescent products formed from NA and DA after freeze-drying and formaldehyde treatment were examined in different parts of the spinal cord under the fluorescence microscope (Falck, Hillarp, Thieme & Torp, 1962; Hillarp, Fuxe & Dahlström, 1966; Corrodi & Jonsson, 1967). Histochemical estimations were made on coded preparations and were independent of the biochemical results. The ability to accumulate DA and NA formed from L-DOPA in the spinal cord cranial and caudal to a transection was also analysed histochemically after pretreatment with FLA-63 or H 44/68 (Table 3).

The statistical significance of the differences observed was calculated by analysis of variance with one criterion of classification or by Student's *t* test (Snedecor & Cochran, 1967). If the *P* value was higher than 0·05, a difference was considered to be not significant (N.S.).

### *Experiments on the L-DOPA action in the spinal cord*

For the number of experiments and treatments, see Tables 3–6. The hind limb flexor reflex activity of spinalized animals is facilitated by increased NA receptor stimulation as obtained, for example, after injection of L-DOPA (Carlsson, Magnus-

TABLE 1. *Effect of FLA-63 on the noradrenaline concentrations in the rat spinal cord normally and after transection*

| Treatment | Cranial part of the spinal cord intact *in vivo* | Caudal part of the spinal cord intact *in vivo* | Cranial part of the spinal cord sectioned *in vivo** | Caudal part of the spinal cord sectioned *in vivo** |
|---|---|---|---|---|
| No drugs | 100·0 (9) | 100·0 (9) | 111·1 (7) | 99·0 (7) |
| FLA-63 (25 mg/kg i.p. 4 h before death) | 35·5 (9) | 36·7 (9) | 34·6 (7) | 82·0 (7) |

Percentage values (means) of those in the intact, untreated group. Normal noradrenaline concentrations in the spinal cord: cranial part 0·18 μg/g, caudal part 0·32 μg/g. Number of experiments in parentheses. Variance within groups 1152.1013 with 55 degrees of freedom. * Spinal cord transection about 6 h before death.

TABLE 2. *Effect of FLA-63 on the fluorescence intensity of noradrenaline (NA) nerve terminals in the intact spinal cord and the transected spinal cord of rats*

| Treatment | Fluorescence intensity* Cranial spinal cord | | | Caudal spinal cord | | | Effect on fluorescence intensity in NA nerve terminals compared with sectioned and intact controls not treated with drug |
|---|---|---|---|---|---|---|---|
| Transected spinal cord | | | | | | | |
| No drug treatment | 3+ | (8) | | 3+ | (8) | | |
| FLA-63 (25 mg/kg) | 1+ | (5) | 2+ (5) | 2+ | (1) | 3+ (9) | Disappearance of fluorescence only cranial to the transection |
| FLA-63 (2×20 mg/kg) | 1/2+ | (4) | 1+ (7) | 2+ | (3) | 3+ (8) | As above |
| Intact spinal cord | | | | | | | |
| FLA-63 (25 mg/kg) | 1+ | (4) | 2+ (5) | 1+ | (3) | 2+ (6) | Disappearance of fluorescence in both cranial and caudal part |
| FLA-63 (2×20 mg/kg) | 1/2+ | (2) | 1+ (6) | 1/2+ | (3) | 1+ (5) | As above |

The spinal cord transection was carried out on the previous evening. FLA-63 (25 mg/kg) was given intraperitoneally 4 h before death, or in two doses of 20 mg/kg, 5 and 2 h before death. * Semiquantitative estimation of fluorescence intensity: very strong=4+; strong=3+; moderate=2+; weak=1+; very weak 1/2+. Number of rats in parentheses.

son & Rosengren, 1963; Andén, Jukes & Lundberg, 1966; Andén, Corrodi, Fuxe & Hökfelt, 1967). The effect of different pretreatments with FLA-63 on this L-DOPA action was investigated in rats which had been spinalized 2–1·5 h before testing. The effect of the tyrosine hydroxylase inhibitor H 44/68 (Andén, Corrodi, Dahlström, Fuxe & Hökfelt, 1966; Corrodi & Hanson, 1966) and of the uptake-storage blocking compound, reserpine, on the change in flexor reflex activity induced by L-DOPA was studied in the same way. The reactivity of the NA-sensitive effector cells after the various pretreatments was tested by administration of clonidine (0·4 mg/kg i.p.), which appears directly to stimulate the NA receptors (Andén, Corrodi, Fuxe, Hökfelt, Hökfelt, Rydin & Svensson, 1970). The increase in the flexor reflex by pinching a hind leg was semiquantitatively estimated by an investigator who was unaware of the treatment. After the functional tests, the caudal part of the spinal cord and the whole brain were taken for biochemical analysis of NA and DA as described above (Table 5).

## Results

### *Effect of FLA-63 on the concentrations of NA and DA in the intact and transected spinal cord*

Four hours after treatment with FLA-63 (25 mg/kg i.p.), the NA concentration was reduced in both the cranial and caudal half of the intact spinal cord by approximately 65%. In the transected spinal cord, the loss of NA was similar cranial to the lesion to that observed in the intact spinal cord. Caudal to the cut, however, there was no significant reduction of the NA content. The concentration of NA in the caudal part was significantly ($P<0·02$) higher than in the cranial half of the sectioned cord and in the caudal half of the intact cord. Histochemically, depletion of fluorescence in the NA terminals after FLA-63 was obvious throughout the intact spinal cord but only in the cranial half of the transected spinal cord. After two doses of FLA-63 (20 mg/kg) 5 and 2 h before death, the NA depletion was even greater in the cranial part of the transected cord but was still insignificant caudal to the transection (Tables 1 & 2).

After treatment with three doses of FLA-63 for 15 h and spinal cord transection 1·5 h before death, the NA in the caudal half of the spinal cord and in the brain was reduced to 17 and 7% of the normal value respectively (Table 5). The activation and fluorescence spectra were characteristic for NA when peaks could be recor-

TABLE 3. *Effect of DOPA on the fluorescence intensity of noradrenaline (NA) nerve terminals in the spinal cord of spinalized rats pretreated with FLA-63 to deplete the stores of NA in the central nervous system*

| Treatment | Fluorescence intensity* | | | | Effect on fluorescence intensity compared with FLA-63 and H 44/68 treated controls |
|---|---|---|---|---|---|
| No drug treatment | | | | 3+ (9) | |
| DOPA | | | | 3+ (6) | |
| FLA-63 | 1/2+ (4) | 1+ (8) | | | |
| FLA-63+DOPA | | 1+ (4) | 2+ (9) | | Only partial repletion |
| H44/68 | 1/2+ (8) | 1+ (3) | | | |
| H44/68+DOPA | | | | 3+ (8) | Repletion to normal concentrations |

FLA-63 (20 mg/kg) was given intraperitoneally twice 6 and 3 h before killing. H 44/68 (250 mg/kg) was given intraperitoneally twice 8 and 4 h before killing. All the rats were spinalized 2 h before killing and L-DOPA (100 mg/kg, intraperitoneally) was injected 1 h before death. * For details, see Table 2. Identical results were obtained caudal and cranial to the transection.

ded. Simultaneously, the spinal cord was found to contain DA in amounts which were significantly higher ($P<0.01$) than in the controls (Table 5). The activation and fluorescence spectra were identical with those of authentic DA after treatment with FLA-63 but not in the controls. The DA content observed was, however, smaller than that of the missing NA. In the histochemical experiments (Tables 2, 3) the depletion of fluorescence was smaller than expected from the biochemically determined loss of NA (Tables 1, 5).

The difficulty in replacing the NA by DA was also seen in histochemical experiments (Table 3). The NA terminals in the acutely transected spinal cord were depleted of fluorescence to almost the same degree after two doses of FLA-63 as after two doses of H 44/68. Furthermore, the injection of a small dose of L-DOPA caused a complete restitution of the fluorescence in the NA terminals of the animals treated with H 44/68, but only a partial reappearance in those treated with FLA-63.

### *Experiments on the L-DOPA action in the spinal cord*

After pretreatment with the monoamine oxidase inhibitor nialamide, in a dose which was inactive by itself, injections of L-DOPA caused an increase in the hind limb flexor reflex of spinal rats. The increase was maximal 30 min after the injection. When FLA-63 (25 mg/kg i.p.) was administered 30 min before L-DOPA, there was no obvious change in the L-DOPA-induced flexor reflex increase (Table 4). Immediately after administration of FLA-63 athetoid movements of the hind legs with a tendency to increase in flexor reflex activity were observed but this effect had always disappeared before the L-DOPA injection.

Pretreatment with two doses of FLA-63 (2 × 25 mg/kg i.p., 7 and 3 h before test) produced a clear-cut reduction of the increase in flexor reflex activity observed after L-DOPA treatment (Table 4). Pretreatment with the tyrosine hydroxylase inhibitor H 44/68 (100+250 mg/kg i.p. 12 and 6 h before test) did not produce the same pronounced decrease in the L-DOPA response. To judge from the chemical results in this and a previous study (Andén, *et al.*, 1966) the reduction in the concentrations of NA after FLA-63 and H 44/68 were approximately equal before the L-DOPA injection.

Treatment with FLA-63 does not inhibit DA-β-hydroxylase activity for more than 8 h (Corrodi, personal communication). Therefore, three doses of FLA-63

TABLE 4. *Effect of acute pretreatment with FLA-63 and of chronic pretreatment with FLA-63 or H 44/68 on the DOPA-induced increase in hind limb flexor reflex activity**

| Drug treatment† | Magnitude of increase in flexor reflex‡ | | | | |
|---|---|---|---|---|---|
| DOPA 25 | | | | | 3+ (26) |
| DOPA 5 | | | | 2+ (27) | |
| FLA-63 25 | | 1/2+ (5) | | | |
| FLA-63 25+DOPA 25 | | | | 2+ (1) | 3+ (8) |
| FLA-63 25+DOPA 5 | | | 1+ (2) | 2+ (9) | |
| FLA-63 2×25 | 0 (6) | | | | |
| FLA-63 2×25+DOPA 25 | | | 1+ (10) | 2+ (7) | |
| FLA-63 2×25+DOPA 5 | | 1/2+ (11) | 1+ (3) | | |
| H 44/68 100+250 | 0 (6) | | | | |
| H 44/68 100+250+DOPA 25 | | | | 2+ (4) | 3+ (8) |
| H 44/68 100+250+DOPA 5 | | 1/2+ (6) | 1+ (9) | | |

* Midthoracic spinal cord transection 2 h before testing. † Drug treatment before testing: nialamide (100 mg/kg i.p.) 2·5 h to all rats, L-DOPA (i.v.) 15–30 min, FLA-63 (25 mg/kg i.p.) 1 h, FLA-63 (2×25 mg/kg i.p.) 7+3 h, H 44/68 (100+250 mg/kg i.p.) 12+6 hours. ‡ Semiquantitative estimation: 3+ =very strong, 2+ =strong, 1+ =moderate, 1/2+ =small, 0=normal=no increase. Number of rats in parentheses.

TABLE 5. *Effect of pretreatment with FLA-63, H 44/68 and reserpine on the DOPA-induced increase in the hind limb flexor reflex activity and on the increase in the concentrations of noradrenaline and dopamine of the caudal half of the spinal cord and of the brain of acutely spinalized rats**

| Drug treatment† | Magnitude of increase in flexor reflex† | | | | Noradrenaline§ | | Dopamine§ | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Caudal spinal cord | Whole brain | Caudal spinal cord | Whole brain |
| DOPA | | | | 3+(19) | 0·510±0·0273 (9) | 0·615±0·0233 (9) | 2·398±0·1367 (9) | 3·478±0·1309 (9) |
| Without DOPA | 0 (18) | | | | 0·528±0·0291 (9) | 0·508±0·0201 (9) | 0·113±0·0403 (9) | 0·768±0·0316 (9) |
| FLA-63×3+DOPA | 1/2+(13) | 1+(6) | 2+(3) | | 0·054±0·0086 (8) | 0·031±0·0034 (8) | 1·498±0·1128 (8) | 1·917±0·1047 (8) |
| FLA-63×3 | 0 (21) | | | | 0·089±0·0122 (8) | 0·034±0·0071 (8) | 0·315±0·0497 (8) | 0·732±0·0342 (8) |
| FLA-63+H 44/68+DOPA | 1/2+(3) | 1+(5) | 2+(12) | 3+(2) | 0·128±0·0160 (8) | 0·132±0·0199 (8) | 1·576±0·1812 (8) | 1·639±0·1467 (8) |
| FLA-63+H 44/68 | 0 (22) | | | | 0·098±0·0171 (8) | 0·063±0·0102 (8) | 0·063±0·0179 (8) | 0·156±0·0081 (8) |
| Reserpine+DOPA | | 1+(3) | 2+(17) | | 0·062±0·0108 (9) | 0·088±0·0126 (9) | 0·927±0·1042 (9) | 1·387±0·1663 (9) |
| Reserpine | 0 (19) | | | | 0·025±0·0087 (9) | 0·023±0·0050 (9) | 0·080±0·0265 (9) | 0·046±0·0096 (9) |
| Reserpine+FLA-63+DOPA | 0 (1) | 1/2+(7) | 1+(12) | 2+(3) | 0·017±0·0044 (8) | 0·012±0·0009 (8) | 1·929±0·2381 (8) | 2·772±0·2932 (8) |
| Reserpine+FLA-63 | 0 (20) | | | | 0·014±0·0028 (8) | 0·008±0·0017 (8) | 0·096±0·0303 (8) | 0·039±0·0113 (8) |
| Reserpine+H 44/68+DOPA | | 1+(2) | 2+(15) | 3+(5) | 0·082±0·0109 (8) | 0·102±0·0097 (8) | 1·313±0·2528 (8) | 1·985±0·3097 (8) |
| Reserpine+H 44/68 | 0 (20) | | | | 0·019±0·0048 (8) | 0·015±0·0046 (8) | 0·075±0·0333 (8) | 0·041±0·0210 (8) |

* Spinal cord transection 1·5 h before testing or death. † Drug treatment before testing or death: nialamide (50 mg/kg i.p.) 1·5 h to all rats, L-DOPA (37·5 mg/kg i.v.) 30 min, FLA-63×3 (40+25+25 mg/kg i.p.) 15+7+2 h, FLA-63+H 44/68 (40+250 mg/kg i.p.) 15+7 h, reserpine (10 mg/kg i.p.) 7 h, reserpine+FLA-63 (10+25 mg/kg i.p.) 7+2 h, reserpine+H 44/68 (10+250 mg/kg i.p.) 7+2 h. † Semiquantitative estimation: 3+=very strong, 2+=strong, 1+=moderate, 1/2+=small, 0=normal=no increase. Number of rats in parentheses. § Values (mean±S.E.M.) in μg/g 30 min after the L-DOPA injection. Number of experiments in parentheses.

(40+25+25 mg/kg i.p.) were given in order to cause a maximum depletion of the NA stores. The NA concentration was lowered to about the same level if H 44/68 was given instead of the two later doses of FLA-63 (Table 5) which is more than can be achieved with H 44/68 alone (cf. Persson & Waldeck, 1970a & b). The increase in flexor reflex activity induced by L-DOPA was substantially diminished after pretreatment with three doses of FLA-63 (Table 5). It was larger, though still far from normal, after pretreatment with FLA-63 plus H 44/68. After reserpine pretreatment, the flexor reflex response to L-DOPA injection was clearly weaker than normal but greater than after three doses of FLA-63 (Table 5).

Treatment with L-DOPA increased the NA about equally after pretreatment with FLA-63 plus H 44/68 or with reserpine but seemed to lower the NA after pretreatment with three doses of FLA-63 ($P<0.05$ in the spinal cord; Table 5). The rises in DA induced by L-DOPA were of the same magnitude in the three groups mentioned but were significantly lower than in the controls.

Since the condition of the rats deteriorated after repeated doses of the synthesis inhibitors, experiments were done in which FLA-63 or H 44/68 was given acutely before L-DOPA to spinal rats pretreated with reserpine (Table 5). The reserpine pretreatment should also cause a greater depletion and prevent the newly formed amines from being trapped in the granules (Corrodi, Fuxe & Hökfelt, 1966). Administration of L-DOPA produced increases in the hind limb flexor reflex in both cases but it was clearly larger in the group treated with H 44/68 than in that treated with FLA-63. The formation of NA was apparently inhibited rather efficiently by FLA-63 but not by H 44/68 (Table 5).

In order to test the sensitivity of the effector cells to NA, the NA receptor stimulating agent clonidine was given to rats pretreated similarly to some of those in Table 5. The clonidine-induced increases were about equal in all groups and were maximal after about 30 min (Table 6). The condition of the animals treated with repeated doses of FLA-63 and H 44/68 was not as good as in the other groups and several deaths occurred.

## Discussion

The DA-β-hydroxylase inhibitor FLA-63 caused in 4 h a decrease of about 65% of the NA concentration throughout the intact spinal cord and also cranial to the section of a transected cord. The same rate of disappearance has been observed after treatment with another DA-β-hydroxylase inhibitor, sodium diethyldithiocarbamate, whereas only a 50% reduction of the NA content was observed 4 h after treatment with tyrosine hydroxylase inhibitors (Andén *et al.*, 1966; Andén,

TABLE 6. *Effect of pretreatment with FLA-63, H 44/68 and reserpine on the clonidine-induced increase in hind limb flexor reflex activity of acutely spinalized rats**

| Drug treatment† | Magnitude of increase in flexor reflex‡ | | |
|---|---|---|---|
| Nialamide+clonidine | 1+ (4) | 2+ (4) | |
| FLA-63×3+nialamide+clonidine | 1+ (5) | 2+ (4) | 3+ (1) |
| FLA-63+H 44/68+nialamide+clonidine | 1+ (6) | 2+ (3) | |
| Reserpine+nialamide+clonidine | 1+ (5) | 2+ (3) | |

* Spinal cord transection 2 h before testing. † Drug treatment before testing: nialamide (50 mg/kg i.p.) 1·5 h, clonidine (0·4 mg/kg i.p.) 30 min, FLA-63 (40+25+25 mg/kg i.p.) 15+7+2 h, FLA-63+H 44/68 (40+250 mg/kg i.p.) 15+2 h, reserpine (10 mg/kg i.p.) 7 hours. ‡ Semiquantitative estimation: 3+=very strong, 2+=strong, 1+=moderate. Number of rats in parentheses.

Fuxe & Hökfelt, 1966 & 1967, Andén, 1967). This difference between tyrosine hydroxylase and DA-β-hydroxylase inhibitors has also been observed in the brain (Persson & Waldeck, 1970a & b).

The decrease in the NA concentration was clearly dependent on the normal impulse flow since only a small, statistically insignificant reduction was seen caudal to a transection. All the inhibitors of the NA and 5-hydroxytryptamine synthesis studied in this way need nerve impulses for a substantial depletion of the amine stores (Andén *et al.* 1966; Andén, Fuxe & Hökfelt, 1966 & 1967). On the other hand, drugs blocking the uptake-storage mechanism or displacing the endogenous amines cause a reduction in the amine concentration which is almost completely independent of the nerve impulses (Andén, Fuxe & Hökfelt, 1966 & 1967; Andén, Fuxe & Henning, 1969).

Treatment with FLA-63 produced a slight accumulation of DA in the spinal cord, although it was not as great as the loss of NA. The same phenomenon has been observed in the brain after treatment with disulfiram and diethyldithiocarbamate (Goldstein & Nakajima, 1967; Carlsson, Fuxe & Hökfelt, 1967). The spinal cord has the advantage that there are no DA neurones in this region. The DA accumulated was probably present in the NA terminals since the histochemical method reflects total NA and DA contents and the histochemically observed fluorescence did not appear to be depleted to the same extent as the concentration of NA was lowered in the biochemical experiments. Another indication of the difficulty for DA to accumulate in the usual NA stores was the small restoration of fluorescence after injection of L-DOPA to rats treated with FLA-63 but not with H 44/68. NA has a higher affinity than DA for the subcellular particles of peripheral NA nerves (Musacchio, Kopin & Weise, 1965; Musacchio, Fischer & Kopin, 1966).

The main purpose of this investigation was to study the mode of action of L-DOPA in the spinal cord, which contains NA but not DA nerve terminals and, therefore, might be considered to have NA but not DA receptors. Acute pretreatment with FLA-63 did not appreciably change the L-DOPA response. It may be either that the DA formed from L-DOPA can act like the NA on the receptors of the effector cells or that the DA can displace and release the NA stored in the granules.

Displacement of NA by DA formed from L-DOPA was indicated from the results obtained with rats treated with repeated doses of FLA-63 since there was a slight further reduction of NA after L-DOPA treatment in these rats. Also, without DA-β-hydroxylase inhibition, DA can partly replace the NA in peripheral nerves shortly after the administration of large doses of this amine (Harrison, Levitt & Udenfriend, 1963). Also, the smaller response to L-DOPA after depletion of the NA stores by repeated doses of H 44/68 may indicate that displacement is of functional importance. It must be remembered that all these experiments were performed after a partial inhibition of the monoamine oxidase by nialamide. The displacing agents may act like reserpine on the granular uptake mechanism (Andén, 1964; Carlsson, 1965) and may, thus, result in a condition analogous to the so-called inverse reserpine syndrome after monoamine oxidase inhibition. Therefore, conclusive evidence with regard to involvement of a displacing mechanism can be obtained only if L-DOPA is given without monoamine oxidase inhibition and such experiments are in progress.

The functional role of the DA formed from L-DOPA on the NA receptors can be analysed from the data obtained after depletion of the NA stores. To judge from the results of the clonidine experiments, the sensitivity of the effector cells to NA was not changed after the various pretreatments. The response to L-DOPA treatment was clearly smaller in rats pretreated with FLA-63 than in those treated with H 44/68 or reserpine. This finding may indicate that the formation of a small amount of NA is of a greater functional significance than the larger formation of DA but that the DA may have a weak activity on the NA receptors. There was, however, a slight displacement of NA in the FLA-63 experiments. Furthermore, the animals were not in an ideal condition after treatment with repeated doses of FLA-63 but this was also true for treatment with FLA-63 plus H 44/68. These difficulties were overcome in the experiments where FLA-63 or H 44/68 was given immediately before administration of L-DOPA to reserpine pretreated rats. Again, the increase in the flexor reflex response after L-DOPA treatment was much weaker in the FLA-63 treated group and the formation of NA but not DA was inhibited. Therefore, it appears that NA is much more efficient than DA in stimulating the central NA receptors. The DA may have about the same action on the central as on the peripheral NA receptors, that is, 20–100 times weaker.

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