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THE INFLUENCE OF APOMORPHINE AND TRICYCLIC ANTIDEPRESSANT DRUGS ON THE LEVEL OF SEROTONIN AND ITS METABOLITE IN RAT BRAIN

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Abstract—Brain levels of serotonin and 5-hydroxyindoleacetic acid were measured in rats after combined treatment with apomorphine and tricyclic antidepressants. The apomorphine-induced elevation of 5-hydroxyindoleacetic acid was prevented by tertiary but not by secondary antidepressants. The results are discussed in relation to the possible participation of serotonin in the mechanism of action of apomorphine.

Tricyclic antidepressants (TAD) inhibit the reuptake of both noradrenaline and serotonin (5-HT) [1–8]. This effect seems to depend on the substitution of nitrogen in the aminopropyl chain [2, 6–8]. Monomethylated TAD in general do not inhibit 5-HT reuptake [1, 7–10].

Apomorphine selectively stimulates dopamine receptors in the central nervous system [11, 12]. The effect seems to involve both noradrenergic [13–15] and serotoninergic neurons [16]. The elevation of the 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in rat brain under the influence of apomorphine observed previously by us [16] and recently confirmed by Schell-Krüger and Hasselager [17] suggests increased utilization of 5-HT.

The inhibition of the reuptake of 5-HT limits the contact of 5-HT with monoamine oxidase (MAO) and consequently the level of 5-HIAA should decrease. If apomorphine increases 5-HT utilization, actual inhibition of 5-HT uptake may inhibit the apomorphine-induced increase in the level of 5-HIAA in brain.

This paper discusses the influence of TAD on the levels of 5-HT and 5-HIAA in the brain of normal and apomorphine-treated rats.

MATERIALS AND METHODS

Male and female Wistar rats, weighing 120–160 g, were used.

The level of 5-HT and 5-HIAA in a single brain was determined by the spectrofluorometric method of Maickel *et al.* [18] and that of Miller *et al.* [19].

Apomorphine at a dose of 5·0 mg/kg was administered subcutaneously 30 min after intraperitoneal injection of different doses of TAD. The animals were killed 30 min later. The dose of apomorphine used was chosen from previous experiments [16, 20] as the lowest effective dose elevating the level of 5-HIAA in whole brain as well as in separate brain areas in about 80 per cent of the experiments. The rise in 5-HIAA after a higher dose of apomorphine, 25·0 mg/kg, was not generally higher than after 5·0 mg/kg of the drug and a dose of 1·0 mg/kg did not affect the level of 5-HIAA in more than half of the experiments. The hydrochlorides of the following substances were used: apomorphine (McFarlane), amitriptyline (Saroten, Lundbeck), chlorimipramine (Anafranil, Ciba–Geigy), imipramine (Polfa) and nortriptyline (Lundbeck). The compounds were dissolved in physiological saline in the constant volume of 4 ml/kg. Statistical analysis was performed using Student's *t*-tests.

RESULTS

None of the TAD compounds, at the doses used, influenced the level of 5-HT in rat brain (Table 1). The level of 5-HIAA was significantly lowered by imipramine (10·0 mg/kg), amitriptyline (25·0 mg/kg), chlorimipramine (5·0 mg/kg) and nortriptyline (10·0 mg/kg). The level of 5-HIAA was not altered after administration of other doses of these compounds or after desipramine injection (Table 2).

The administration of apomorphine (5·0 mg/kg) raised the level of brain 5-HT in only one experiment, but increased significantly the level of 5-HIAA in all experiments (Table 2).

The increase in 5-HIAA level induced by apomorphine was inhibited by all doses used of amitriptyline, chlorimipramine and imipramine, as well as by the higher of two doses of desipramine and nortriptyline (Table 2). Nortriptyline (5·0 mg/kg) and desipramine (10·0 mg/kg) had no influence on the increase in 5-HIAA level resulting from the action of apomorphine.

Nortriptyline at a dose of 5·0 mg/kg depressed significantly the apomorphine induced rise in the level of 5-HT. The level of 5-HT after combined treatment with chlorimipramine and apomorphine was significantly lower than the control value.

DISCUSSION

No changes were observed in the level of 5-HT after TAD administration. This result is in agreement with recently reported data [6, 10, 21–24]. The TAD-induced increase in 5-HT, observed sporadically [25–27], has been found to appear usually after administration of higher doses than those used by us.

Table 1. The influence of apomorphine and TAD on the level of 5-HT in the rat brain

TAD (mg/kg)	5-HT (ng/g ± S.E.M.) Saline	TAD + saline	Saline + apomorphine	TAD + apomorphine
Imipramine (5·0)	607 ± 39·2	608 ± 43·9	711 ± 34·1	690 ± 62·2
Imipramine (10·0)	580 ± 10·2	591 ± 13·3	611 ± 14·2	596 ± 21·3
Imipramine (25·0)	607 ± 39·2	483 ± 48·8	711 ± 34·1	779 ± 41·4
Amitriptyline (5·0)	566 ± 34·3	571 ± 24·3	611 ± 6·9	718 ± 16·8§
Amitriptyline (10·0)	530 ± 14·1	512 ± 26·7	512 ± 19·6	557 ± 21·5
Amitriptyline (25·0)	566 ± 34·3	635 ± 19·7	611 ± 6·9	614 ± 25·5
Chlorimipramine (5·0)	566 ± 34·3	639 ± 14·1	611 ± 6·9	620 ± 23·0
Chlorimipramine (10·0)	530 ± 14·1	538 ± 27·3	512 ± 19·6	427 ± 21·8†
Desipramine (10·0)	577 ± 17·2	564 ± 15·7	593 ± 9·1	555 ± 15·4
Desipramine (25·0)	607 ± 39·2	619 ± 32·1	711 ± 34·1	680 ± 45·4
Nortriptyline (5·0)	543 ± 8·2	555 ± 9·3	583 ± 6·4‡	548 ± 7·0‡
Nortriptyline (10·0)	530 ± 14·1	481 ± 25·4	512 ± 19·6	475 ± 25·2

* $P < 0.05$ † $P < 0.02$ ‡ $P < 0.01$ § $P < 0.001$.

Each group involved 6–8 animals. For statistical analysis groups treated with apomorphine or TAD were compared with saline treated group while groups injected with TAD and apomorphine were compared with respective apomorphine pretreated group.

Table 2. The influence of apomorphine and TAD on the level of 5-HIAA in the rat brain

TAD (mg/kg)	5-HIAA (ng/g ± S.E.M.) Saline	TAD + saline	Saline + apomorphine	TAD + apomorphine
Imipramine (5·0)	563 ± 11·7	559 ± 19·4	736 ± 16·9§	613 ± 20·1§
Imipramine (10·0)	571 ± 23·9	454 ± 22·6‡	677 ± 17·7‡	586 ± 32·0*
Imipramine (25·0)	563 ± 11·7	554 ± 10·3	736 ± 16·9§	592 ± 21·5§
Amitriptyline (5·0)	535 ± 14·7	465 ± 29·9	735 ± 42·3§	450 ± 20·1§
Amitriptyline (10·0)	529 ± 22·8	545 ± 19·7	648 ± 16·0§	542 ± 21·1‡
Amitriptyline (25·0)	535 ± 14·7	392 ± 19·2§	735 ± 42·3§	445 ± 39·7§
Chlorimipramine (5·0)	535 ± 14·7	369 ± 25·2§	735 ± 42·3§	429 ± 23·9§
Chlorimipramine (10·0)	529 ± 22·8	478 ± 22·5	648 ± 16·0§	474 ± 17·2§
Desipramine (10·0)	647 ± 24·1	641 ± 20·2	796 ± 18·1‡	771 ± 20·6
Desipramine (25·0)	563 ± 11·7	549 ± 12·5	736 ± 16·9§	661 ± 17·3§
Nortriptyline (5·0)	575 ± 12·3	582 ± 10·6	655 ± 12·1§	626 ± 7·1
Nortriptyline (10·0)	529 ± 22·8	443 ± 23·4*	648 ± 16·0§	524 ± 25·0‡

For explanations see Table 1.

The level of endogenous 5-HIAA was decreased by some doses of imipramine, amitryptyline and chlorimipramine, as found by other authors [26–29]. The decrease in the level of this 5-HT metabolite may result from the inhibition of 5-HT uptake into the neuron and the diminished contact with MAO. It is also probable that accumulation of endogenous amine at the synapse, secondary to reuptake inhibition, results in a diminution of synthesis and turnover rate of brain 5-HT through negative feedback [7, 22, 23, 26, 27, 29, 30, 31]. We failed to observe any significant decrease in the level of 5-HIAA after administration of higher doses of imipramine and chlorimipramine, which is particularly effective in the inhibition of 5-HT uptake [3, 29, 30, 32, 33]; this observation holds true also for the lower doses of amitriptyline used. The level of 5-HIAA was decreased following the administration of 10 mg/kg of nortriptyline. According to the literature [1, 7–10] this compound should have practically no influence on serotonin neurons. Neither in our own research nor in that of other workers did we find any changes in the level of 5-HIAA due to the administration of the other monomethylated derivative, desipramine [10, 21]. It seems that the ability of TAD to impede 5-HT uptake mechanisms depends on the doses of the drugs rather than nitrogen substitution of the aminopropyl chain. This idea is supported by data in the literature [2, 3, 6, 25, 33].

In our opinion, the apomorphine-induced increase in brain 5-HIAA as well as the increased level of 5-HT which was observed in some experiments is due to an intensified turnover of brain 5-HT [16]. It is suggested by both the apomorphine-induced increase of 5-HT depletion in the brain of rats in which tryptophan hydroxylase activity was blocked, as well as the acceleration of 5-HIAA disappearance in the brain of pargyline pretreated rats.*

TAD-induced inactivation of 5-HT reuptake into the neuron should counteract the rise of brain 5-HIAA induced by apomorphine. The inhibition of apomorphine-induced increase in the level of 5-HIAA was produced by administration of tertiary TAD, amitriptyline, chlorimipramine and imipramine. The lower doses of nortriptyline and desipramine used failed to produce any changes in 5-HIAA level increased by apomorphine. The inhibitory effect of the higher doses of these drugs on the apomorphine-induced changes in 5-HIAA level suggests again that the 5-HT uptake mechanism can be inhibited also by secondary TAD, provided these compounds are administered at relatively high doses.

* M. Grabowska, unpublished data.

The increase in the rate of 5-HT turnover in brain produced by apomorphine seems to have a definite pharmacological effect [16, 20], which is revealed by locomotor stimulation in rats [16, 34]. We found earlier that the monomethylated TAD, desipramine and nortriptyline had no effect on apomorphine-induced stimulation of locomotor activity in rats. On the other hand, this stimulation was weakened by the dimethylated TAD imipramine and amitriptyline [35], compounds that are said to have a more selective effect on serotonin neurons. Chlorimipramine did not inhibit the locomotor stimulation caused by apomorphine. These results indicate that chlorimipramine administered together with apomorphine decreased significantly the level of brain 5-HT and at the same time inhibited significantly the apomorphine-induced increase in the level of 5-HIAA. The biochemical changes resulting from combined treatment with apomorphine and chlorimipramine suggests both deficiency as well as excess of 5-HT at the serotonin receptor. In the latter case, the lack of inhibitory effect of chlorimipramine on apomorphine-induced locomotor stimulation in rats still remains unclear. It might be that the increased utilization of 5-HT caused by apomorphine is compensated by the TAD-induced decrease of 5-HT synthesis and turnover rate resulting from excess of neuromediator at the synapse. This explanation is supported by the fact that among the tertiary TAD chlorimipramine is particularly active in reducing the impulse activity of brain raphe neurons [31, 36].

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REFERENCES

1. J. J. Schildkraut, S. M. Schanberg, G. R. Bresse and I. J. Kopin. *Biochem. Pharmac.* **18**, 1971 (1969).
2. S. B. Ross and A. L. Renyi. *Eur. J. Pharmac.* **7** 270 (1969).
3. M. H. Kannengiesser, P. Hunt and J. P. Reynaud. *Biochem. Pharmac.* **22**, 73 (1972).
4. A. Carlsson, K. Fuxe and U. Ungerstedt. *J. Pharm. Pharmac.* **20**, 150 (1968).
5. L. Gyermed. *Int. J. Neurobiol.* **9**, 95 (1966).
6. A. Carlsson, H. Corrodi, K. Fuxe and T. Hökfelt. *Eur. J. Pharmac.* **5**, 357 (1969).
7. J. Schubert, H. Nybäck and G. Sedvall. *J. Pharm. Pharmac.* **22**, 136 (1970).
8. K. Fuxe and U. Ungerstedt. *Eur. J. Pharmac.* **4**, 135 (1968).
9. D. Palaic, I. H. Page and P. A. Khairallach. *J. Neurochem.* **14**, 63 (1967).
10. R. Samanin, D. Ghezzi and S. Garattini. *Eur. J. Pharmac.* **20**, 281 (1972).
11. N. E. Andén, A. Rubenson, K. Fuxe and T. Hökfelt. *J. Pharm. Pharmac.* **19**, 627 (1967).
12. A. Ernst, *Psychopharmacologia* **10**, 316 (1967).
13. J. Maj, M. Grabowska and L. Gajda. *Eur. J. Pharmac.* **17**, 208 (1972).
14. T. Persson and B. Waldeck, *Acta physiol. scand.* **78**, 142 (1970).
15. T. Persson and B. Waldeck, *Eur. J. Pharmac.* **11**, 315 (1970).
16. M. Grabowska, L. Antkiewicz, J. Maj and J. Michaluk. *Pol. J. Pharm. Pharmac.* **25**, 29 (1973).
17. J. Scheel-Krüger and E. Hasselager. *Psychopharmacologia* **36**, 189 (1974).
18. R. P. Maickel, R. H. Cox, Jr., J. Saillant and F. P. Miller. *Int. J. Neuropharm.* **7**, 275 (1968).
19. F. P. Miller, R. H. Cox, W. R. Snodgrass and R. P. Maickel. *Biochem. Pharmac.* **19**, 435 (1970).
20. M. Grabowska, J. Michaluk and L. Antkiewicz. *Eur. J. Pharmac.* **23**, 82 (1973).
21. A. Tagliamonte, P. Tagliamonte, J. Perez-Cruet and G. L. Gessa. *J. Pharmac. exp. Ther.* **177**, 475 (1971).
22. H. Corrodi and K. Fuxe. *J. Pharm. Pharmac.* **20**, 230 (1968).
23. H. Corrodi and K. Fuxe, *Eur. J. Pharmac.* **7**, 56 (1969).
24. R. W. Fuller, R. J. Schaffer, B. W. Roush and B. B. Molloy. *Biochem. Pharmac.* **21**, 1413 (1972).
25. G. F. Oxenkrug. *Farmakol. Toksikol.* **35**, 150 (1972).
26. A. E. Halaris, R. A. Lovell and D. X. Freedman. *Biochem. Pharmac.* **22**, 2200 (1973).
27. J. Bruinvels. *Eur. J. Pharmac.* **20**, 231 (1972).
28. M. da Prada and A. Pletscher. *J. Pharm. Pharmac.* **18**, 628 (1966).
29. K. Modigh. *J. Pharm. Pharmac.* **25**, 926 (1973).
30. J. Meek and B. Werdinius. *J. Pharm. Pharmac.* **22**, 141 (1970).
31. M. H. Sheard, A. Zolovick and G. K. Aghajanian. *Brain Res.* **43** 690 (1972).
32. S. B. Ross, A. L. Renyi and S. O. Ögren. *Eur. J. Pharmac.* **17**, 107 (1972).
33. A. Carlsson. *J. Pharm. Pharmac.* **22**, 729 (1970).
34. M. Grabowska and J. Michaluk. *Pharmac. Biochem. Behav.* **2**, 263 (1974).
35. M. Grabowska, L. Antkiewicz and J. Michaluk. *Pol. J. Pharm. Pharmac.* **26**, 411 (1974).
36. G. J. Bramwell. *Br. J. Pharmac.* **44**, 345 (1972).