TRICYCLIC ANTIDEPRESSIVES—A POSSIBLE MODE OF ACTION

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The catecholamines have important functions, probably as neurotransmitters, in the central nervous system. One of the catecholamines, dopamine, is possibly involved in those systems regulating the locomotor activity of animals.[1-5]

Dopamine is formed in certain parts of the central nervous system, for instance in the substantia nigra of the reticular formation, in relatively large amounts. The biosynthesis and metabolic inactivation of dopamine are presented in Fig. 1.

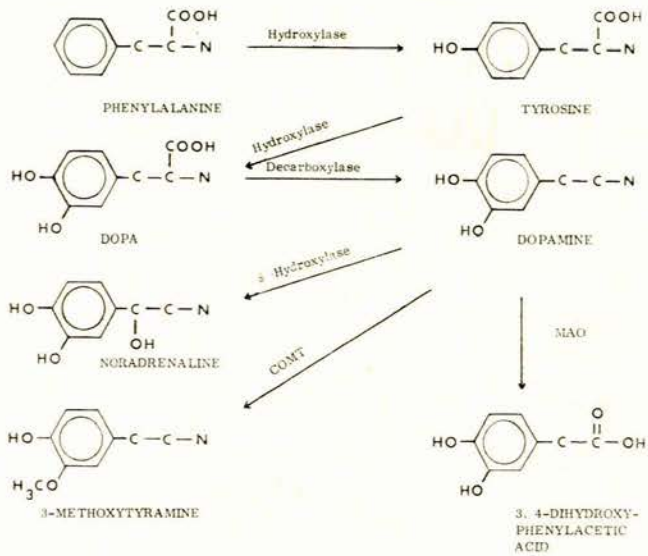

Fig. 1. The biosynthesis and metabolic inactivation of dopamine. Phenylalanine is converted to tyrosine and dihydroxyphenylalanine (DOPA) by hydroxylation. Decarboxylation of DOPA, the direct precursor of dopamine, leads to the formation of the latter substance. Decarboxylation presumably takes place in the nerve-endings where dopamine is 'stored'. Most of the formed dopamine is bound to protein; in the bound form it cannot be broken down by the enzyme monoamine oxidase (MAO). MAO occurs in the mitochondria and metabolizes unbound ('free') monoamines such as dopamine and noradrenaline to the corresponding organic acids. Dopamine is released from its bound form by nerve impulses and is then able to diffuse into the synaptic cleft and act as an agonist on the dopamine receptors. In the synaptic cleft dopamine is inactivated by the enzyme catechol-O-methyltransferase (COMT).

It is known that drugs causing a depletion of dopamine in the central nervous system, such as reserpine, etc., and drugs presumably acting as competitive antagonists on the dopamine receptors like chlorpromazine, etc., inhibit locomotor activity.[6-10] These groups of drugs may be used as sedatives or 'major tranquillizers'. On the other hand an enhancement of locomotor activity is exhibited by drugs with the following mechanisms of action:

(*a*) close structural analogues of dopamine, presumably acting as agonists directly on dopamine receptors, like amphetamine, etc. ('direct action') (Fig. 2);

(*b*) drugs causing a rapid release of bound dopamine, thereby causing an increase in the dopamine concentration in the vicinity of the dopamine receptors which are then stimulated by dopamine itself, like benzylamphetamine or cocaine ('indirect action');

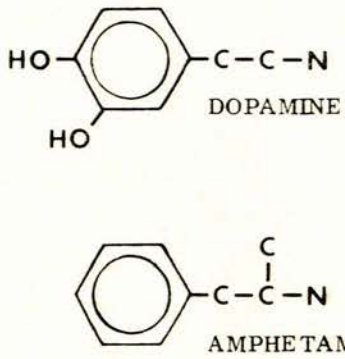

Fig. 2. Dopamine and its analogue amphetamine.

(*c*) 'precursors' of dopamine like L-DOPA, which is metabolized to dopamine in the central nervous system, thereby causing an increase in the concentration of dopamine; and

(*d*) drugs that inhibit the metabolic inactivation of dopamine, like monoamine-oxidase inhibitors (MAO inhibitors).

Some stimulant drugs presumably act by more than one of the above mechanisms, for instance it has been shown that methylphenidate (Ritalin) and phenmetrazine (Preludin) act by mechanisms (*a*) and (*b*).[1] Many drugs acting by the above mechanisms are or have been therapeutically used as 'central stimulants' or 'antidepressants'.

The role of dopamine as a neurotransmitter and the mechanisms of action of the abovementioned groups of drugs are schematically given in Fig. 3.

Dopamine is released from its protein-bound form ('stores') by nerve impulses along the presynaptic fibre. The released dopamine diffuses via the cell membrane to the synaptic cleft and acts as an agonist on specific dopamine receptors on the postsynaptic nerve cell, causing a stimulus leading to an impulse along the postsynaptic nerve fibre and, eventually, eliciting the effect—an increase in locomotor activity. Some of the dopamine in the synaptic cleft is presumably inactivated by the enzyme catechol-O-methyltransferase (COMT), and the rest diffuses back into the presynaptic cell, possibly transported across the cell membrane by an active transport mechanism. In the presynaptic cell most of the dopamine is again bound to protein, but a small amount is inactivated by monoamine oxidase (MAO) in the mitochondria. There is, apparently, an equilibrium between bound and free dopamine in the presynaptic cell.

Experimental evidence for the role of dopamine in locomotor activity was mainly obtained on mice, rats and guinea-pigs, using photocell activity cages to measure the activity. It was shown that intraperitoneal injection of amphetamine or cocaine in these animals gives an increase of locomotor activity. After pretreatment of the animals with reserpine, which depletes the catechola-

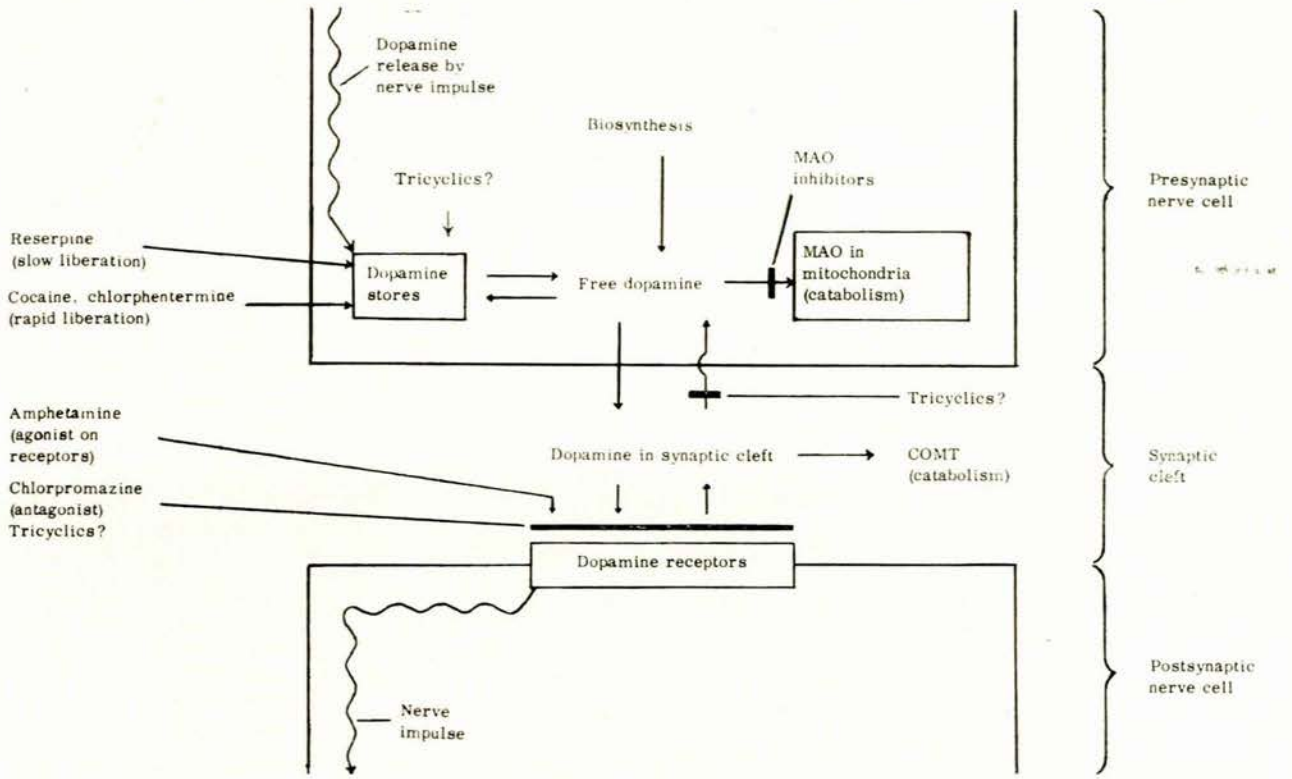

Fig. 3. Schematic presentation of the role of dopamine and the mechanism of action of various stimulant and sedative drugs.

mines in the central nervous system, amphetamine gives the same increase in locomotor activity as before, but cocaine has no effect. This indicates that amphetamine is not dependent on the presence of dopamine for its action and acts directly as an agonist on the dopamine receptors, but that cocaine cannot give an effect in the absence of dopamine and therefore presumably acts by a rapid release of dopamine from the protein-bound stores. Amphetamine may be called a 'direct dopaminergic drug' and cocaine an 'indirect dopaminergic drug'.

The phenothiazines like chlorpromazine and other competitive antagonists of noradrenaline on the alpha-receptors in the periphery all have a sedative action if they are lipophylic enough to cross the blood-brain barrier.[1,7] These compounds all antagonize the increase in locomotor activity caused by amphetamine or cocaine and may be assumed to act by competitive antagonism on the dopamine receptors, since dopamine and noradrenaline are structurally very closely related.[1,7,11]

The tricyclic antidepressives are structurally closely related to the phenothiazines, yet they appear to have opposite effects on the central nervous system.

In experiments on isolated organs it could be shown that the tricyclic antidepressives sensitize the organs to the action of noradrenaline[12-14] whereas the phenothiazines act as antagonists.[15] In high concentrations, however, the tricyclic antidepressives act as competitive antagonists of noradrenaline.[16,17] The sensitization has been ascribed to an inhibition of an active transport mechanism responsible for the uptake of noradrenaline into nerve cells.[18-22] The present investigation was carried out to determine whether the mechanism of the tricyclic antidepressives in the central nervous system could be related to their sensitization towards catecholamines as shown for noradrenaline.

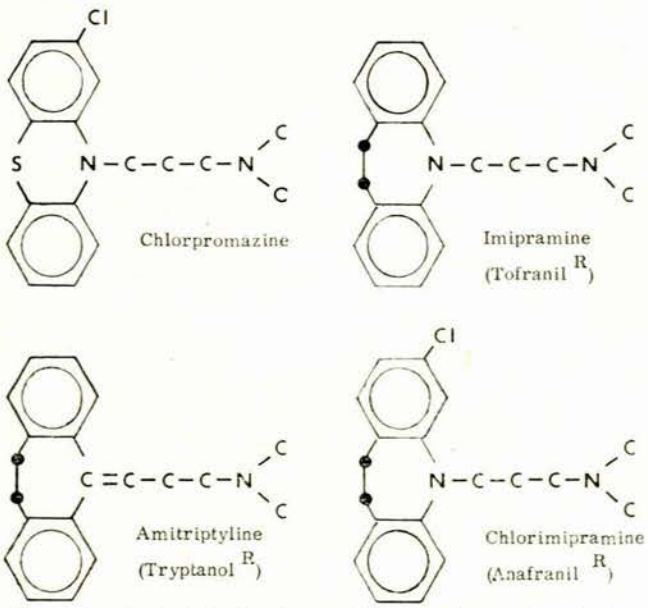

Fig. 4. Structural relationship between chlorpromazine and the tricyclic antidepressives.

EXPERIMENTS

Photocell activity cages were used to determine the action of the drugs used on the locomotor activity of Swiss-Webster mice. The cages measured 18 cm. × 65 cm. and had two parallel light beams, focused on two cadium sulphide photo-electric cells, over the width of the cage, 12·5 cm. from each end. The interruption of the light beams was registered with cumulative recorders as described in various publications.[3,11,23] The recorders record each interruption of a light beam by an upward movement of 1 mm. of the recording pen on a sooted kymograph drum. The drum rotated at 0·5 mm./min. and every 30 seconds the pen returned to the zero position, so that each vertical line on the registration represents the number of light beam interruptions during a 30-second period. The height of these lines is a measurement of the locomotor activity of the animals.

Groups of 6 mice were used for each experiment and all drugs were injected intraperitoneally in isotonic solutions. The concentrations of the drugs in solution were such that 0·01 ml./G body-weight was always injected. Control experiments with isotonic sodium chloride injections were done in all cases.

In experiments where mice were pretreated with an MAO inhibitor, the compound pargyline (Eutonyl) was used. Three injections, each of 500 μmol./kg., were given, at 12-hr intervals. The last of these 3 injections was given 1 hr before treatment with the tricyclic antidepressive.

RESULTS

None of the tricyclic antidepressants investigated gave an increase of locomotor activity on its own. These compounds, however, antagonize the increase in locomotor activity caused by direct or indirect dopaminergic drugs (Fig. 5).

The antagonistic effects as described above correspond to the antagonistic effects produced by phenothiazines like chlorpromazine. However, when an inhibitor of MAO is given before administering one of the tricyclic compounds, the latter causes an increase in locomotor activity (Fig. 6). (The dose of the MAO inhibitor is such that no increase in locomotor activity is found when the inhibitor is given alone.) This increase is not found with the phenothiazines.

The tricyclic antidepressants of which these aspects were investigated are imipramine (Tofranil), desmethylimipramine (Pertrofan), chlorimipramine (Anafranil), amitriptyline (Tryptanol), nortriptyline (Aventyl), protriptyline (Concordin), opipramol (Insidon), meclofenoxate (Lucidril), dibenzepine (Noveril) and trimipramine (Surmontil). Of these the dimethyl derivatives such as imipramine, amitriptyline and especially chlorimipramine were very active in causing an increase in locomotor activity of the mice. These substances also acted more rapidly than the other substances. The mono- and trimethyl derivatives are less active, whereas dibenzazepine derivatives with the side-chain in positions other than 1 (such as opipramol, meclofenoxate, etc.) are inactive as far as increased locomotor activity is concerned.

DISCUSSION

The results indicate that the tricyclic antidepressant drugs investigated have at least a dual mode of action in the central nervous system. They exhibit an antagonistic action towards dopaminergic drugs corresponding to the antagonistic action of the phenothiazine derivatives. It is assumed that this antagonism would correspond to a sedative effect. It must be pointed out that the antagonistic action of the tricyclic antidepressives is found at high doses, much higher than doses needed for a similar antagonism by, for instance, chlorpromazine. Since the tricyclic antidepressants are structurally closely related to the phenothiazines, it is possible that the antagonistic

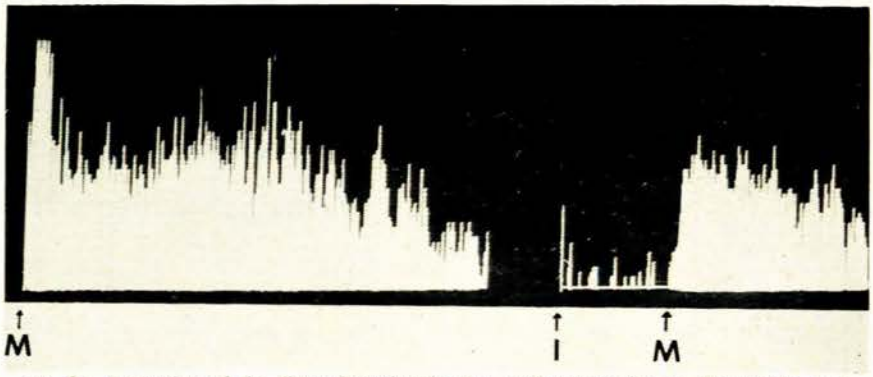

Fig. 5a. Antagonism of the direct dopaminergic drug methamphetamine by the tricyclic antidepressive imipramine (Tofranil). M = methamphetamine 15 μmol./kg.; I = imipramine 100 μmol./kg.

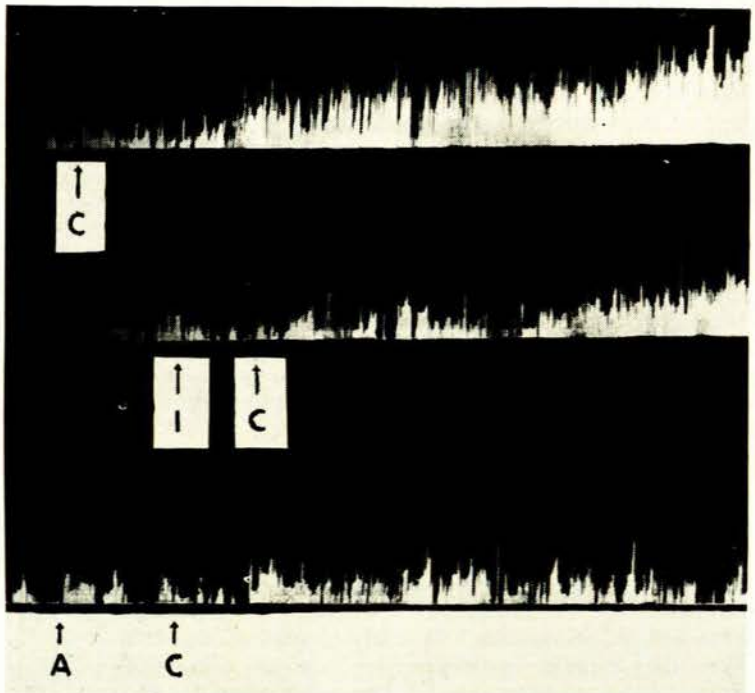

Fig. 5b. Antagonism of the indirect dopaminergic drug chlorphentermine by the tricyclic antidepressives amitriptyline (Tryptanol) and imipramine (Tofranil). C = chlorphentermine 400 μmol./kg.; I = imipramine 100 μmol./kg.; A = amitriptyline 100 μmol./kg. Note in both cases the reduction of the effect of the agonists by previous administration of a tricyclic antidepressive drug.

action for both these groups of drugs may be ascribed to the same mechanism. Ariëns and Simonis[24] have postulated that competitive antagonism of noradrenaline on alpha-sympathetic receptors by chlorpromazine may be ascribed to an affinity of the phenothiazine nucleus of chlorpromazine to an 'additional' or 'accessory' receptor area. Noradrenaline, as mentioned earlier, is also antagonized by the tricyclic antidepressives in higher concentrations. There is a difference between the spatial structure of the dibenzazepine nucleus of the tricyclic antidepressives and the phenothiazine nucleus of the phenothiazine derivatives. If the nucleus is the main contributory factor towards affinity on the accessory receptor area it seems likely that for affinity to the alpha-receptors of noradrenaline as well as for affinity to the dopamine receptors, the phenothiazine nucleus has the more advantageous spatial structure.

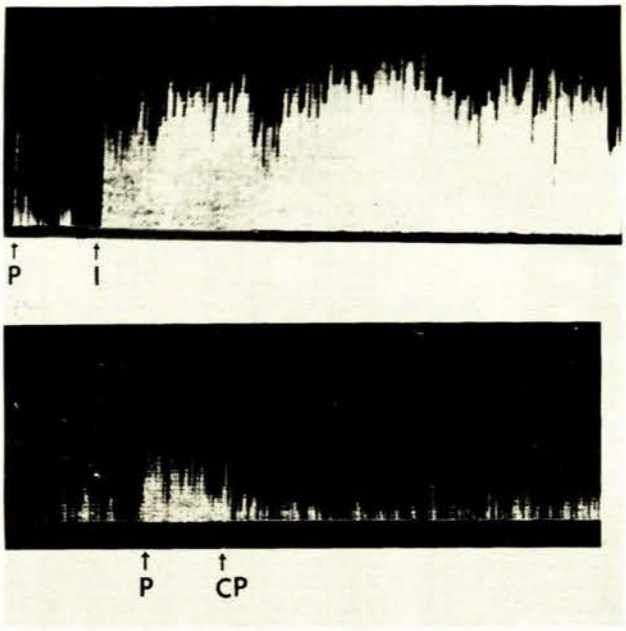

Fig. 6. Increase in locomotor activity caused by the tricyclic antidepressive imipramine (Tofranil) after pretreatment with the MAO inhibitor pargyline (Eutonyl). Note that a similar increase is not produced by the phenothiazine compound chlorpromazine. P = the last injection of pargyline 500 μmol./kg.; I = imipramine 100 μmol./kg.; CP = chlorpromazine 7·5 μmol./kg.

Although the tricyclic antidepressant drugs do not cause an increase in locomotor activity on their own, unlike the other groups of antidepressant drugs mentioned earlier, they cause a substantial increase in the locomotor activity of the animals after pretreatment with an inhibitor of MAO (Fig. 6). Reserpine, which is known to cause a depletion or slow release of dopamine from its stores, also causes an increase in locomotor activity after pretreatment with an inhibitor of MAO, since, under these circumstances, the slowly released dopamine is not broken down by MAO and the increase of the dopamine concentration in the synaptic cleft is large enough to cause an increase in locomotor activity.[3]

The tricyclic antidepressives, however, are unable to deplete the dopamine stores like reserpine does when given at regular intervals over a period of time. This is shown by the fact that the effect produced by indirect dopaminergics is not altered by pretreatment with the antidepressives in this way. It seems, therefore, that if the tricyclic antidepressives act by releasing dopamine slowly from its stores, the mechanism of release is different from that exhibited by reserpine.

A second probable mechanism of action to be considered is an inhibition of re-uptake in the nerve ending, as has been described for the tricyclic antidepressives in sympathetic nerve endings.[18-22] If this is the primary mechanism of action, one would expect a strong potentiation of the indirect dopaminergic drugs in the presence of the tricyclic antidepressives, since the concentration of dopamine in the synaptic cleft would then be increased by the blockade of the re-uptake mechanism. Such a potentiation, however, does not take place. Another finding not favouring this mechanism of action is that the tricyclic antidepressives increase locomotor activity after inhibition of MAO. The enzyme MAO is found in the nerve cell as such and a blockade of the re-uptake of released dopamine into the nerve cell by a tricyclic antidepressive would therefore rather tend to lessen the importance of MAO. The fact that inhibition of MAO is a prerequisite for an increase in locomotor activity by the tricyclic antidepressives tends to support the view that the latter compounds act by liberating dopamine from its stores. Since the tricyclic antidepressives do not cause an increase in locomotor activity when given alone, it appears that the liberation of dopamine is not very fast and that MAO can cope with the inactivation of the liberated amine. Once MAO is inhibited, however, the concentration of unbound dopamine in the presynaptic nerve cell becomes so high that dopamine diffuses into the synaptic cleft, causing an increase in locomotor activity.

SUMMARY

The tricyclic antidepressives seem to have at least a dual mode of action in the central nervous system. They antagonize the effect of dopaminergic drugs, but after pretreatment with MAO inhibitors they cause an increase in locomotor activity.

The increase in locomotor activity is presumably caused by a slow liberation of dopamine from its protein-bound stores. The mechanism of this liberation does not appear to be the same as the liberation caused by reserpine.

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