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INTERACTION OF D-CYCLOSERINE WITH THE ACTION OF SOME MONOAMINE OXIDASE INHIBITORS

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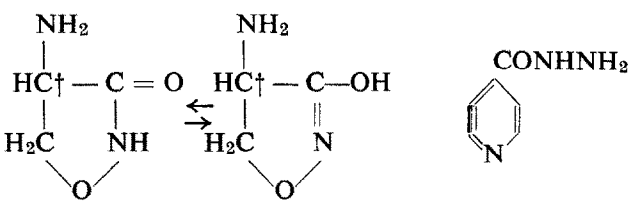
Abstract—The effect of D-cycloserine pretreatment on monoamine oxidase inhibition by isoniazid (INH), pheniprazine and tranylcypromin was determined in rat liver and brain. The animals were killed 6 hr after the D-cycloserine and 2 hr after the administration of monoamine oxidase inhibitors. With the doses used the behaviour was markedly influenced by cycloserine pretreatment only in the case of pheniprazine. D-cycloserine itself exerted a slight, but significant, inhibition of the enzyme activity in liver. Cycloserine antagonised the inhibition of monoamine oxidase by isoniazid both in liver and brain. Contrary to isoniazid, the inhibitory action of pheniprazine and tranylcypromin was potentiated in both tissues. It was concluded that cycloserine probably forms a hydrazone with isoniazid, while potentiation of pheniprazine and tranylcypromin effects may be due to an unspecific reaction with the protein moiety of the enzyme.

THE antibiotic cycloserine, 4-amino-isoxazolidin-3-on, has been reported to have psychotropic properties, the quality and intensity of which differ in its optical isomers.[1] For the treatment of pulmonary tuberculosis and some infections of the uro-genital tract the D-isomer, which has the lowest toxicity,[2] is generally used.

The antidepressant action of D-Cycloserine (Cs) was successfully used in the treatment of depressive tuberculous patients[3] and later also in non-tuberculous depressive subjects.[4] In a detailed comparative study we have shown that, contrary to the L-isomer, D-cycloserine is without any significant depressant action. Long-term administration of higher doses showed that because of its effects, especially due to frequent emotional disturbances, D-Cs resembles rather the action of psychic energizers than that of imipramine-like drugs.[5] Comparing the effect of Cs-isomers on monoamine oxidase activity (MAO) we have found that there is a difference in the action of individual isomers *in vitro* as well as *in vivo*. In liver DL-Cs exerts the strongest effect.[2]

In the present paper we studied the effect of D-Cs pretreatment on MAO inhibition by several drugs of different structure as shown below.



cycloserine isoniazid

(tautomeric forms; † denotes asymmetric carbon).

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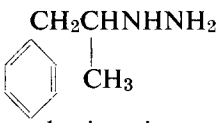
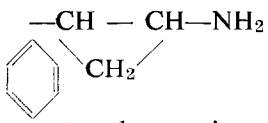


pheniprazine



tranylcypromin

There were several facts which stimulated this investigation.

1. In a series of clinical experiments designed to antagonize the psychotropic effect of a single dose of L-Cs, the main property of which is strong sedation with some antidepressant component,[5] the psychic changes were considerably protracted by iproniazid, isonicotinyl-isopropylhydrazine, a potent MAO inhibitor.[6] 2. Cs and isoniazid interfere with the vitamin B_6 metabolism.[7] 3. D-Cs and isoniazid have some neurotoxic properties which are common to both drugs in many respects.[8, 9] 4. Isoniazid, which is isonicotinyl hydrazine, is usually used for the treatment of tuberculosis in combination with D-Cs. It also has some MAO inhibiting properties.[10] 5. It has been proved that oxo- compounds such as pyruvic acid and alpha-ketoglutaric acid antagonize the convulsive action of isoniazid, probably due to the formation of hydrazones which were even detected in urine.[11, 12] As shown above, it is assumed that cycloserine could have a similar effect as an oxo-compound. 6. Horita and Matsumoto[13] found that sodium pyruvate can antagonize the inhibitory action of pheniprazine (β-phenylisopropylhydrazine) but not that of tranylcypromin (*trans*-2-phenylcyclopropylamine). This action of pyruvate on MAO inhibitors is limited only to the compounds which have a free hydrazine group.[14]

METHOD

A total of 408 albino Wistar rats, weighing ~ 150—200 g each, were used in this investigation which consisted of a series of individual experiments. For each experiment the rats were divided into four groups, each group containing 6 rats. The group serving as a control received physiological saline, the second group D-Cs and the third group received one of the three MAO inhibitors. The fourth group was given MAO inhibitor after pretreatment with D-Cs. For the estimation of MAO activity in liver and brain different animals were used. Each experiment was carried out three times except when assaying the pheniprazine effect on brain, where seven experiments with different Cs–pheniprazine ratios were carried out. In this paper, only the results of two experiments are reported in detail. Behaviour was evaluated only according to rough criteria, i.e. the overall impression. All drugs were injected intraperitoneally. The usual experimental design was as follows: the control animals were injected with physiological saline twice, (at 0 and 4 hrs.). The second group received only cycloserine at the 0 hr and the saline was injected after 4 hr. The third group was given physiological saline at 0 hr. one of the MAO inhibitors after 4 hr. The fourth group was injected at 0 hr with Cs and after 4 hr with the MAO inhibitor. All animals were sacrificed by decapitation 6 hr after the first injection. The sequence of the sacrificing was as follows: a, b, c, d; d, a, b, c; c, d, a, b; and so on. The fourth hr for the injection of MAO inhibitors was chosen because according to previous experience the action of Cs on MAO activity developed satisfactorily as late as 6 hr. after its administration.[5] The solutions were prepared in such a way that for a rat weighing 200 g a 2 ml volume of each drug was used. All MAO inhibitors were dissolved in distilled water and Cs in a $\frac{1}{3}$ saturated solution of $NaHCO_3$. Saline was used as a control because the final

pH value of the Cs solution was close to seven. All solutions were prepared shortly before administration. Pheniprazine and tranylcypromin were used as the respective hydrochlorides. In the reported experiments 1·5 g D-Cs, 150 mg INH, 50 mg pheniprazine and 50 mg tranylcypromin/kg were administered.

After sacrificing, livers or brains were rapidly removed and ground with a teflon homogeniser in water to a 33 or 50% w/v aqueous homogenate, respectively. All procedures were carried out in an ice bath. One-ml aliquots of the homogenates were incubated with serotonin creatinine sulphate for 60 min at 37° in a shaking water bath. Essentially a modification of the method of Sjoerdsma *et al.*[15] was used. Instead of measuring the remaining serotonin we determined the 5-hydroxylindoleacetic acid formed, using the method of Udenfriend *et al.*[16] This is more specific since part of the serotonin disappears from the incubation medium by other processes than deamination. A detailed description of the modified assay is given elsewhere.[17] The detected amount of 5-hydroxyindoleacetic acid was calculated per 100 mg of dry wt. The results were evaluated by the t-test as the percentage when the mean of all controls (54 animals for liver and 48 for brain) was taken as 100 per cent.

RESULTS

Behaviour

Within 30 min of receiving a single dose of D-Cs (1·5 g/kg), the majority of animals showed a strongly decreased response to external stimuli such as pain, touch and noise, a decreased agressivity, and the defense reaction was characterized by a decrease of spontaneous motility. These symptoms gradually intensified and the animals later suffered from a considerable drowsiness. The muscle tension was highly decreased and the general picture in some animals resembled a state of narcosis. After the 4th hr these symptoms were still well developed.

When 150 mg/kg isoniazid, was administered alone the animals rapidly became excited, restless and suffered from tremor with occasional convulsions and produced painful sounds. The defence reaction towards touch was strongly increased.

The pheniprazine injection (50 mg/kg) produced only a moderate excitement manifested first of all by an increased exploratory amphetamine-like activity.

The excitation produced by 50 mg/kg tranylcypromin was very strong, as has been described repeatedly.[18]

The injection of MAO inhibitor changed the behaviour after D-Cs in each case. The overall picture of INH effects did not significantly change by Cs pretreatment. Only the aggressivity to touch seemed somewhat decreased.

A very marked change in behaviour occurred with pheniprazine. The drowsiness evoked by Cs rapidly diminished after Cs pretreatment and shortly a strong excitement was observed, by far exceeding that after the injection of pheniprazine alone. The animals also suffered from strong jumping convulsions. This state lasted till the end of the experiments.

The tranylcypromin injection suppressed completely the effect of Cs on behaviour and there was no evident behavioral change in the tranylcypromine-injected animals as compared with those having in addition Cs pretreatment.

Inhibition of MAO

In Figs. 1 and 2 the result of MAO inhibition in liver and brain, are given. In liver, the inhibition of MAO by D-cycloserine, though small, is statistically significant

($P<0{\cdot}01$). The value in Fig. 1 represents the results obtained in 54 animals, while those for other drugs and their combination with Cs were obtained only in 18 animals in each case.

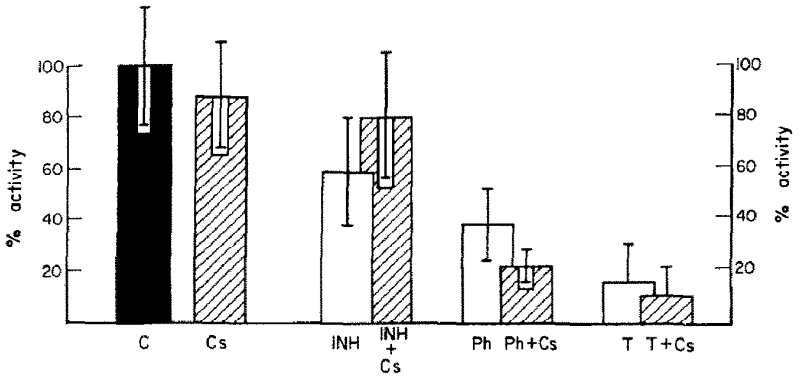


FIG. 1. Activity of MAO in liver after the pretreatment with D-Cs. C = control, Cs = D-Cs, INH = isonazid, P = pheniprazine, T = tranylcypromin. Cs was injected i.p. 4 hr prior to isoniazid, pheniprazine and tranylcypromin. Animals were sacrificed 6 hr after the first injection. For control and D-Cs values 48 rats were used each, in other cases 18 rats were used.

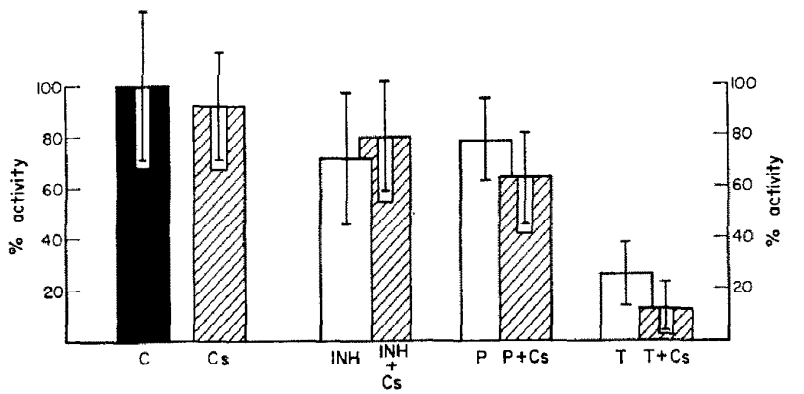


FIG. 2. Activity of MAO in brain after the pretreatment with D-Cs. Abbreviations and technical data as in FIG. 1.

The suppression of the enzyme activity by INH alone is suprisingly intense (41·6 per cent on an average). The reversion of the inhibition by Cs-pretreatment is not complete, but nevertheless very remarkable, and as apparent from Table 1, the change is statistically significant. Contrary to isoniazid, the effects of pheniprazine and tranylcypromin were influenced in an opposite way, there being a significant increase of inhibition in the case of pheniprazine. Due to a very strong inhibition of activity by tranylcypromin alone, the change was not significant, though the average value of activity decreased from 14·6 to 9·0 per cent when the inhibition reached a 100 per cent value in many animals.

In brain (Fig. 2) the MAO inhibition is in general very similar to that in liver. The absolute value of inhibition, however, is smaller with all drugs used. The degree of

TABLE 1 SIGNIFICANCE OF VALUES OF MAO ACTIVITY AFTER INDIVIDUAL MAO INHIBITORS.*

	Significancy value	D-Cs	INH	INH + Cs	Ph	Ph + Cs	T	T + Cs
Liver	Related to control	0·01	0·001	0·01	0·001	0·001	0·001	0·001
	Related to MAO inhibitor	—	0·01		0·01		0·3	
Brain	Related to control	0·2	0·001	0·05	0·05	0·01	0·001	0·001
	Related to MAO inhibitor	—	0·4		0·1		0·05	

* The results were evaluated according to the t-test and the values of significance are expressed as $P<$.

MAO inhibition by Cs alone is also smaller due to a higher variability and does not reach the level of significancy ($P<0·2$). The action of isoniazid is quite evident also in the brain. While in liver it was reversed by Cs-pretreatment by about 50 per cent, in brain the reversion amounts only to 34 per cent. After Cs pretreatment the inhibitory action of pheniprazine and tranylcypromin was greatly increased. The change of activity was significant with the latter at the level $P<0·05$, and with pheniprazine it closely approached this level. Besides these changes activity is also apparent from change of average values.

Apart from the experiments presented we made preliminary trials to determine the influence of Cs pretreatment in other combinations, changing the dose of pheniprazine and D-Cs and also the interval between their administration. We used the following combinations: (a) 1 g D-Cs + 20 mg pheniprazine; (b) 1·5 g D-Cs + 20 mg pheniprazine (3 hr after Cs); (c) 1 g D-Cs + 50 mg pheniprazine (3 hr after Cs); (d) 1·5 g D-Cs + 50 mg pheniprazine (3 hr after Cs). With all these combinations there occurred a considerable potentiation of pheniprazine inhibition of MAO, even when 20 mg pheniprazine alone did not produce significant inhibition in brain (within 2 hr).

DISCUSSION

In the reported experiments D-Cs was administered in doses exceeding several times that of MAO inhibitors. All tested drugs penetrate into the brain readily, especially D-Cs. In experiments with mice it was shown[18] that after an oral dose of 300 mg of D-Cs the highest concentration in liver and brain was achieved already within the first 30–60 min. Afterwards the level decreased progressively at a relatively rapid rate. After 3 hr no Cs could be detected in brain, while in liver and blood the concentration still amounted to 10–20 per cent of the original values. The much higher concentrations and different animal species used in the present work make it possible to obtain high Cs levels which may lead to the direct interaction of both administered drugs. There is some indirect evidence that Cs accumulates in tissues or is metabolised to unknown substances. The optimum recovery of a single dose of D-Cs does not exceed 70 percent within 24–48 hr regardless of the animal species or the mode of administration.

The second drug was applied 4 hr after Cs administration. At that time animals were still very strongly influenced by Cs and if they were not treated with MAO inhibitors the state of severe drowsiness lasted till the end of experiment, i.e. 6 hr. The injection of either drug, however, very rapidly abolished the sedative effect of Cs.

Isoniazid and tranylcypromin themselves caused strong excitation so that the influence of Cs pretreatment was not reliably recognizable. This phenomenon seems to be greatly influenced by the concentrations used. The potentiating action of Cs on pheniprazine-induced changes was easily recognizable since in the doses used it caused only slight excitation.

Even when Cs and MAO were not applied simultaneously, it may be suggested from the resulting changes of MAO inhibition that the interaction has hardly the nature of a stechiometric reaction. The mode of Cs administration in our experiments does not rule out that the effective compound is a metabolite of Cs.

From the chemical formula of Cs it is evident that a Cs molecule contains at least two chemically very reactive groups, namely an amino-group in position 4 and a carbonyl one in position 3. Besides Cs can exist in two tautomeric forms (see above).

It has been repeatedly reported[20, 21] that the mechanism of cycloserine action is mainly based on its interference with pyridoxal phosphate. In this reaction, which leads to the formation of a Schiff base, the amino group is involved. On the other hand, however, the mammalian amino oxidase using aromatic amines as a substrate, does not contain pyridoxal phosphate,[22] which together with copper was demonstrated in MAO preparations from beef plasma.[23] It was established that compounds forming a chelate with cupric ions suppress the MAO inhibition by hydrazine inhibitors such as pheniprazine and iproniazid[24] since cupric ions catalyze the decomposition of hydrazine derivatives. It was suggested,[13] and even shown in iproniazid,[25] that the hydrazine liberated during MAO action is the component responsible for MAO inhibition. As Cs does potentiate pheniprazine inhibition it is not possible to conclude that a chelating action with cupric ions participates in its action mechanism.

The carbonyl group in the Cs molecule seems to explain the reduction of MAO inhibition by isoniazid. This reaction should be analogous to that which Horita observed between pheniprazine and pyruvate.[13, 14] Isoniazid forms hydrazones with pyruvic acid and α-ketoglutaric acid and probably with other compounds quite readily.[11, 12] Whether it also forms a hydrazone with cycloserine and whether the resulting compound has some pharmacological activity, has not been proved. The present biochemical results, however, are in good agreement with pharmacological observations indicating that Cs reduces the toxicity of isoniazid.[26, 27]

Contrary to pyruvate, it is probable that Cs does not form a hydrazone with pheniprazine and the resulting potentiation of excitation and MAO inhibition is the result of an increased number of different biochemical side reactions. We do not know whether this fact has at least a slight relationship to the clinical observations that imipramine-like drugs and MAO inhibitors are badly tolerated though both are used for the treatment of depression. It was described that isoniazid, iproniazid and phenylcypromin strongly affect the energy production in the brain and considerably increasing the output of CO_2. This change of the metabolic pattern is more complicated with hydrazine inhibitors as these affect many enzymatic reactions.[28, 29] It is also worthy to note that D-Cs markedly enhances the energy production through the stimulation

of the activity of the tricarbonic acid cycle which reflects very significantly the change in the lactate–pyruvate relationship.[30] Conversely L-Cycloserine increases the production of lactate. From the presented data it follows that the pharmacological action of MAO inhibitors and Cs, as centrally acting agents, is fairly complicated.

Regardless of the effect on behaviour, the comparison of inhibition by pheniprazine and tranylcypromine reveals that the potentiating action on MAO inhibition seems to be independent of the fact that the inhibitor is a hydrazine or a nonhydrazine compound. It is assumed that the principal difference in the mode of action between hydrazine-like inhibitors and tranylcypromin is the fact that the hydrazine inhibits MAO whereas tranylcypromin competes with the neutral substrate for MAO.[31] In our opinion we may conclude that Cs inhibits MAO quite unspecifically when it reacts with a protein moiety of the apoenzyme, as suggested Sazykin.[32] Whether this property can be ascribed only to cycloserine or also to its hypothetic metabolites, remains to be resolved.

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