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HARMALINE AND ITS SULFUR ANALOGUE: INCREASE OF CEREBELLAR CYCLIC GMP AND INHIBITION OF MONOAMINE OXIDASE

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Abstract—Harmaline and its benzo[b]thiophene analogue ("*S*-harmaline") are shown to competitively inhibit monoamine oxidase (MAO). The inhibition was more marked with 5-hydroxytryptamine as a substrate than with tyramine or β-phenylethylamine. After i.p. administration, *S*-harmaline in contrast to harmaline inhibited the MAO in the brain more potently than in the liver. Harmaline produced a greater and longer lasting increase of cGMP in the cerebellum than *S*-harmaline. The *S*-harmaline was also less tremorogenic, thus indicating a connection between cGMP increase and tremor, but not between MAO inhibition and cGMP increase.

Harmaline and related compounds are potent and reversible monoamine oxidase (MAO) inhibitors [1]. In addition, harmaline produces tremor, which seems to be connected with the increase of guanosine 3′,5′-cyclic phosphate (cGMP) in the cerebellum [2–4]. The present communication is concerned with the comparison of a benzo[b]thiophene isostere (*S*-harmaline) of harmaline [5] and harmaline itself (Fig. 1) with regard to their actions on MAO and cGMP.

MATERIALS AND METHODS

The experiments were performed on male albino rats (about 150 g body weight) from the Füllinsdorf stock (SPF). Brains and livers were homogenized in 20 vol. (v/v) potassium phosphate buffer 0.1 M, pH 7.4 in a glass potter and the MAO activity was measured in the total homogenate according to the method of Wurtman and Axelrod [6]. In short, the incubation mixture consisted of 25 μl homogenate, 0.42 M potassium phosphate buffer, pH 7.4 and tyramine, 5-hydroxytryptamine (5-HT) or β-phenylethylamine as a radioactive substrate (2×10^{-4} M) in a total vol. of 300 μl. After preincubation without substrate for 30 min at 37° and incubation for 10 min at 37° the reaction was stopped with 0.2 ml 1 n HCl, extracted with benzene/ethylacetate (1:1, v/v) and the radioactivity measured in a scintillation counter. The linearity was ensured for tissue concentration and for incubation time. For enzyme kinetics, the brains were homogenized in 0.25 M sucrose and the washed mitochondrial fraction was used as an enzyme source [7]. In order to determine the liver/brain quotient, animals were injected i.p. with the drug and decapitated 1 hr later. The MAO activity was then measured in the total homogenate as described above and compared with the activity of untreated animals.

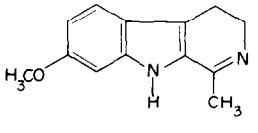
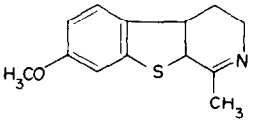


Fig. 1. Harmaline



"*S*-Harmaline"

For the analysis of cGMP the rats were killed by focusing a beam of microwave radiation (2.2 kW; 2.45 GHz; 50 W/cm²) for 7 sec directly on to the head in a manner similar to that described by Guidotti *et al.* [9]. The determination of cGMP in the cerebellum was performed by radioimmunoassay according to the method of Steiner *et al.* [10, 11], as modified by Harper and Brooker [8].

The compounds used were [2-^{14}C]-5-hydroxytryptamine 55 μCi/m-mole, [1,2-^{3}H]tyramine 1.1 Ci/m-mole (both Radiochemical Centre, Amersham, England), β-phenylethylamine [ethyl-1-^{14}C] 51 mCi/m-mole (New England Nuclear), harmaline HCl (Fluka, Buchs, Switzerland), *S*-harmaline (7-methoxy-3,4-dihydro-1-methylbenzothieno[2,3-c]pyridine hydrochloride, kindly supplied by E. Campaigne (Professor of Chemistry, Indiana University, Bloomington, Indiana, U.S.A.). The preparation of the 2′-*O*-succinyl-cGMP and its tyrosinemethylester was performed by Dr. A. Kaiser and the antiserum by Dr. K. Reber.

RESULTS

Single i.p. injections of increasing doses of harmaline induced rapid and progressively larger increases in the content of cGMP in the cerebellum; *S*-harmaline was considerably less potent and its effect of shorter duration (Fig. 2, right). While harmaline produced a 3.5-fold increase at the highest doses tested (50 mg/kg (Fig. 2, left)), *S*-harmaline only doubled the level of cGMP. Higher doses of *S*-harmaline were toxic.

Tremor occurred in all rats receiving 20 or 50 mg/kg of harmaline after a time latency of 3–4 min.

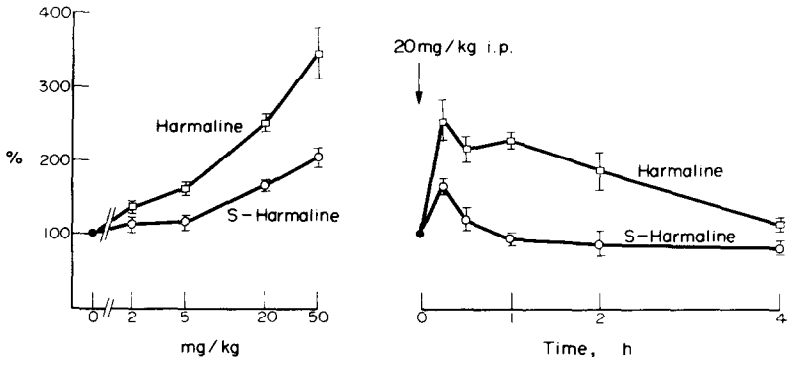


Fig. 2. Effect of harmaline and *S*-harmaline on the level of cGMP in the cerebellum. Harmaline or its *S*-derivate was injected i.p. to rats. The animals were killed by microwave irradiation of the head 15 min after various doses (left) or at different times after the injection of 20 mg/kg (right). Each point is a mean with S.E.M. of at least 8 determinations. The values of cGMP are expressed in per cent of the mean of 16 untreated controls (1.73 ± 0.12 nmole cGMP/g cerebellum).

However, only 50 mg/kg of *S*-harmaline produced some tremor, which was strikingly less strong than with harmaline.

Both compounds inhibited the MAO activity in the liver *in vitro* with similar potency, if 5-HT was used as a substrate. Thus, 50 per cent inhibition was determined between 10^{-9} and 10^{-8} M harmaline or *S*-harmaline. However, with tyramine as substrate, the *S*-harmaline was nearly 100 times more potent than harmaline. With β-phenylethylamine the inhibition was weakest, harmaline hardly reaching 100 per cent inhibition at the very high concentration of 10^{-2} M (Fig. 3).

The nature of MAO inhibition was examined by a Lineweaver–Burk plot using tyramine as substrate. Both inhibitors displayed a competitive mode of inhibition. The K_i-values for the two compounds were similar (4.4 and 5.1 × 10^{-9} M for *S*-harmaline and harmaline, respectively (Fig. 4)).

The inhibition of MAO after i.p. administration of *S*-harmaline and harmaline is shown in Table 1. The ED_{50} of harmaline did not differ in liver or brain thus giving a quotient of 1. However, *S*-harmaline inhibited MAO activity more potently in the brain than in the liver.

DISCUSSION

Harmaline has been shown to activate guanylate cyclase [4] and to increase cGMP in the cerebellum [3, 12–14]. This increase seems to be connected with a tremorogenic action of the drug, since in newborn rats and after treatment with the nicotinamide antagonist 3-acetylpyridine, which destroys the inferior olive, both the tremor and the cGMP increase were absent [12]. The present results support this hypothesis, since *S*-harmaline, an isostere of harmaline, did not induce tremor and produced a much smaller increase in the level of cerebellar cGMP. This might indicate that a certain level of cGMP is necessary to induce tremor.

It has been reported [5] that *S*-harmaline is 50 times more potent as MAO inhibitor in rat liver *in vitro* than harmaline, using tryptamine as substrate. The present experiments confirm and expand this higher potency of the *S*-harmaline with tyramine and β-phenylethylamine as substrate (Fig. 3). However, with 5-HT as substrate practically no difference in the MAO inhibition was seen. This indicates that *S*-harmaline is a less specific inhibitor of the type A MAO than harmaline, which is considered as pure inhibitor of type A [15, 16].

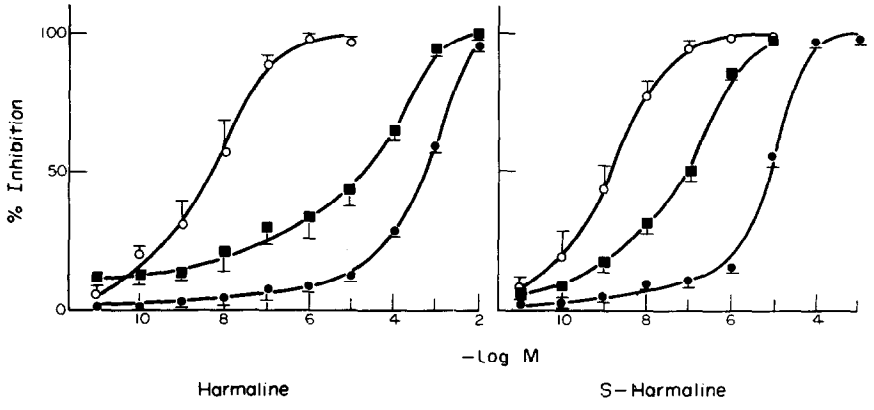


Fig. 3. Inhibition of MAO activity in the rat liver measured with 3 different substrates. MAO activity was measured with 5-HT ○, tyramine ■ and β-phenylethylamine ● as substrate, each with a concentration of 2 × 10^{-4} M. The relative activities without inhibitor were 0.12 ± 0.01, 0.47 ± 0.01, 0.26 ± 0.01 nmole/mg fresh liver/min, respectively. Each point is a mean with S.E.M. of at least 3 triple determinations.

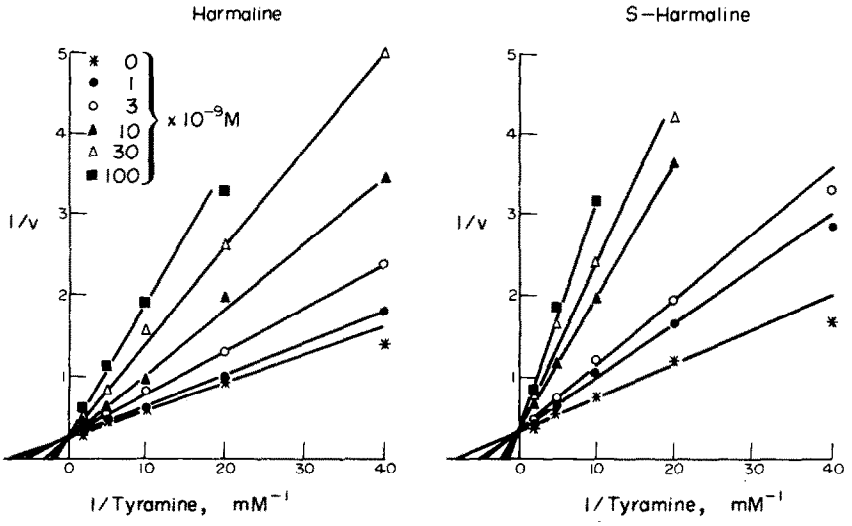


Fig. 4. Lineweaver–Burk plot for MAO inhibition by harmaline and *S*-harmaline. Double reciprocal plot of velocities (v = μmole min^{-1} mg $protein^{-1}$) against concentrations of tyramine at several concentrations of the inhibitors (0–100 × 10^{-9} M). Mitochondrial fraction of rat brain was used as MAO. Each point is a mean of 2 triple determinations.

Table 1. Comparison of MAO inhibition in rat liver and brain after i.p. administration of the inhibitors

		ED_{50} μmole/kg		Ratio
Inhibitor	Substrate	Liver	Brain	ED_{50} Liver/ED_{50} Brain
S-Harmaline	Tyramine	59.0 ± 12	13.0 ± 1.2	4.5 ± 0.6
	5-HT	28.6 ± 10	4.3 ± 1.7	6.6 ± 0.5
Harmaline	Tyramine	56.4 ± 6.2	56.6 ± 11.6	1.0 ± 0.2
	5-HT	25.7 ± 13.0	25.2 ± 8.3	1.0 ± 0.2

One hour after i.p. injection of the inhibitor the MAO activity was measured. Each ED_{50} is a mean with SEM of at least 4 experiments, each with 4–5 different doses (4 animals per dose). The liver/brain quotients were calculated separately for each experiment in order to get a S.E.M. These quotients of the same substrate were significantly ($P < 0.001$) different between the two inhibitors.

The present studies with tyramine as substrate show in a Lineweaver–Burk plot a competitive type of inhibition (Fig. 4). This might partly explain the various degrees of MAO inhibition reported for harmaline in the literature [15, 17], since the substrate concentrations used varied considerably.

In addition the purity of the MAO and the organ source influence the inhibition [18]. Thus MAO is inhibited to a similar extent by harmaline and *S*-harmaline in mitochondrial brain enzyme (Fig. 4), but in liver homogenate, *S*-harmaline is more potent than harmaline (Fig. 3).

The *S*-harmaline has been reported [5] to have more than 200 times higher lipid solubility than harmaline. This might explain the higher liver/brain quotient of the *S*-harmaline (Table 1). In accordance, it has been reported [5] that the administration of the *S*-harmaline to mice resulted in a higher elevation of 5-HT in the brain than with the same dose of harmaline.

In conclusion the cerebellar effect induced by harmaline does not seem to be related to inhibition of MAO, since after i.p. administration, *S*-harmaline is a more potent inhibitor of cerebral than of hepatic MAO, whilst the level of cGMP on the contrary is much more increased by harmaline than by *S*-harmaline. The present results confirm the findings [19] that the tremorogenic activity of harmaline coincides with its capability to increase cerebellar cGMP levels and expand them to an isostere of harmaline which was less tremorogenic and hardly increased the cGMP levels. Thus the present results add evidence to the view indicating a connection between cGMP increase and tremor, but not between MAO inhibition and cGMP increase.

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