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SOME PHARMACOLOGICAL AND BIOCHEMICAL PROPERTIES OF γ-MORPHOLINO-BUTYROPHENONE (NSD 2023), A NEW MONOAMINE OXIDASE INHIBITOR

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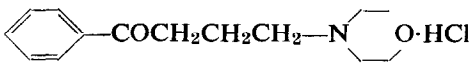
Abstract—Some pharmacological and biochemical properties of γ-morpholino-butyrophenone (NSD 2023) are described. NSD 2023 has a weak sedative effect of 30–60 min duration, and a more pronounced anticonvulsant effect of similar duration, when the substance is administered subcutaneously. In tests for reserpine antagonism (reversal of reserpine induced hypthermia and ptosis), and monoamine oxidase (MAO) inhibition *in vivo*, the substance showed a pronounced effect of at least 4 hr duration. NSD 2023 inhibits mouse brain MAO maximally within 5 min after oral administration. The time required for the reappearance of 50 per cent of inhibited MAO is dose dependent and varies from about 5–17 hr. The maximum inhibition obtainable *in vivo* is about 80 per cent, and the oral dose required to produce 50 per cent of maximum inhibition is about 1·4 mg/kg. *In vitro*, maximum inhibition is about 55 per cent, and the concentration of NSD 2023 required to produce half-maximum inhibition is about 25 μg/ml. Pretreatment of mice with SKF 525A has no apparent effect on the subsequent inhibition of brain MAO by NSD 2023 *in vivo*. Pretreatment of mice with NSD 2023 protects brain MAO against inhibition by phenelzine, pargyline, tranylcypromine, and iproniazid. Similarly, pretreatment with harmaline protects against inhibition by NSD 2023. MAO inhibition by NSD 2023 is only slightly reversed by repeated washes of the mitochondria. A single oral administration of NSD 2023 (50 mg/kg) increases the serotonin content of rat brain by about 50 per cent, but has no significant effect on the concentration of noradrenaline. Pretreatment of rats with NSD 2023 antagonizes the amine depleting action of reserpine. In mice, MAO in brain and kidney is inhibited more than MAO in liver. It is suggested that the antireserpine and anticonvulsant effects of NSD 2023 are not dependent on MAO inhibition as measured using tryptamine or kynuramine as substrate. It is also suggested that the incomplete inhibition of MAO by NSD 2023 reflects the existence of two or more forms of the enzyme.

AT PRESENT several structurally distinct classes of monoamine oxidase (MAO) inhibitors are known,[1, 2] including hydrazines, hydrazides, cyclopropyl amines, guanidines, propargylamines, aminopyrazines, indolealkylamines, and the harmala alkaloids.

In a search for new psychotropic drugs related to γ-amino butyric acid a series of butyrophenone derivatives have been synthesized and pharmacologically investigated in the research laboratories of Ferrosan. One of these substances, γ-morpholino butyrophenone (Fig. 1), was found to be an inhibitor of MAO with properties



NSD 2023

FIG. 1. The structural formula of NSD 2023.

distinguishing it from the known MAO inhibitors, and to possess anticonvulsant and antireserpine activities. It is the purpose of this paper to describe these pharmacological and biochemical properties of NSD 2023.

MATERIALS AND METHODS

Substances

The following substances were generously provided by the manufacturers Pargyline (Abbott), Phenelzine (Lundbeck), Iproniazide and Isocarboxazide (Hoffmann-La Roche), (trans) Tranylcypromine, and SKF 525A (Smith, Kline & French). NSD 2023 was synthesized by S. Hernestam. Other substances were obtained from recognized commercial sources: Reserpine (Serpasil, Ciba), DPN, tryptamine (Sigma), nicotinamide (Light), kynuramine dihydrobromide (Mann).

Animals

Mice were the N.M.R.I. strain weighing from 18–22 g. Rats were the Ferrosan inbred strain, weighing 120–150 g.

Anticonvulsant test

The maximal electroshock seizure (M.E.S.) test, previously described by Christensen *et al.* was used.[3] Mice were fixed by electrode clamps in the skin at the neck and the tail. An electric current (100 V, 20mA, 0·2 sec) induced maximal tonic seizures in 100 per cent of untreated controls. The test compound was administered to 6 animals per dose at different times before the electroshock was applied. The ED_{50} is the dose protecting 50 per cent of the animals from tonic convulsions.

Antagonism of reserpine induced ptosis

The test compound was administered orally (6 mice per dose) 3 times in 24 hr. Three hours after the last treatment reserpine was given (1·0 mg/kg, i.v.). This dose of reserpine induced ptosis in 100 per cent of untreated control animals within 3 hr. The number of mice exhibiting ptosis was evaluated 3 hr after the reserpine injection. In each experiment 6 mice treated with reserpine alone served as controls. The ED_{50} is the dose protecting 50 per cent of the mice against ptosis.

Antagonism of reserpine induced hypothermia

This test was carried out as for ptosis antagonism except that body temperature was measured at different times after reserpine. Room temperature during the test was 19–20°.

Acute toxicity

The test compound was administered s.c. or orally, using 6 mice per dose. The number of mice dying within 24 hr after administration was used to calculate the LD_{50} values.

Statistical analysis

All ED_{50} and LD_{50} values in the animal experiments were calculated by the method of Litchfield and Wilcoxon.[4]

Determination of MAO inhibition in vivo *using tryptamine as substrate*

Mice were killed at various times after administration of inhibitor. The brains were removed immediately and homogenized in 3·0 ml $NaPO_4$ buffer, 25 mM pH 7·4,

containing 2·5 mM ethylenediaminetetra acetate (EDTA-PO_4). The homogenizer was rinsed with an additional 3·0 ml EDTA-PO_4 which was combined with the homogenate in a glass tube.

MAO activity was determined according to Lovenberg *et al.*[5] with the following modifications. The reaction was carried out in a final volume of 400 μl containing 200 μl of brain homogenate, 100 μl of diluted guinea pig kidney supernatant, and 100 μl of a mixture containing PO_4 buffer, tryptamine, nicotinamide and DPN. The latter mixture was added to start the reaction after a 10 min pre-incubation at 37°. After incubating for 30 min at 37°, in air, without shaking, the reaction was stopped by adding 2·0 ml of 0·5 N HCl. The contents of each tube was transferred quantitatively to a 50 ml polypropylene centrifuge tube, using an additional 1·0 ml 0·5 N HCl as a wash. Indole acetic acid was extracted into toluene, back extracted with phosphate buffer, and determined fluorometrically as described by Lovenberg *et al.*[5]

Two mice were used per treatment, and two determinations were made on each homogenate. MAO activities were expressed as arbitrary units per brain. Determined in this way, the amount of MAO per brain is remarkably constant, the standard error being about 2·2 per cent of the mean for the controls. The error in the determination of MAO activity in the brains of mice treated with inhibitors was somewhat greater; for two mice receiving the same treatment the average difference in activity was about 10 per cent of the mean.

It should be pointed out that EDTA increases the MAO activity of mouse brain homogenates 6–8 times, an effect which may be due to increased permeability of synaptosome and mitochondrial membranes.[6,7,45]

Inhibition of MAO in vitro *using tryptamine as substrate*

The method used is the same as above except that 100 μl of brain homogenate (twice the concentration used in the *in vivo* experiments) was pre-incubated for 20 min at 37° with 100 μl of inhibitor solution, after which the reaction was started by adding 200 μl of a mixture containing guinea pig kidney supernatant, PO_4, tryptamine, nicotinamide, and DPN.

Fluorometric determination of MAO using kynuramine as substrate

Kraml has recently shown that 4-hydroxyquinoline, which is formed by the action of MAO on kynuramine, fluoresces intensely in strong base,[8] providing an extremely sensitive method for the determination of MAO. Kraml's method was modified as follows. An aliquot of the mouse brain homogenate described above is diluted 20-fold in 10 mM $NaPO_4$, 100 mM Tris HCl, 1 mM EDTA, pH 8·5 (or in other buffers as described in the text). 200 μl of this diluted brain homogenate is combined with 200 μl kynuramine dihydrobromide 50 μg/ml in distilled water, to start the reaction after 10 min preincubation at 37°. The reaction mixture is incubated for 20 min, at 37°, in air, without shaking. The reaction is stopped by adding 1·0 ml of 1 N NaOH. The fluorescence of 4-hydroxyquinoline is then measured directly (activating and fluorescing wavelengths 318 mμ and 382 mμ, respectively). In separate experiments it was shown that the small amount of protein present in the final assay mixture makes a small contribution to the blank value, but does not quench the fluorescence of 4-hydroxyquinoline. A correction was made for the small quenching produced by NSD 2023.

Preliminary experiments confirmed that phenelzine, pargyline, and tranylcypromine were potent inhibitors of rat liver MAO using kynuramine as substrate. All three substances produced more than 90 per cent inhibition at 0·1 μg/ml. Iproniazid inhibited 95 per cent at 10 μg/ml.

Determination of noradrenaline (NA) and serotonin (5-HT) in rat brain

Noradrenaline and serotonin were extracted simultaneously from rat brain by the method of Shore.[9] Serotonin was determined by the method of Bogdanski *et al.*,[10] and noradrenaline by the method of Shore and Olin,[11] as modified by Schoepke and Wiegand.[12] After addition of freshly prepared alkaline ascorbate, the samples were irradiated with a 60 W tungsten light at a distance of 3–8 cm for 45 min as described by Chin *et al.*,[13] before measuring fluorescence. The NA and 5-HT concentrations are expressed in arbitrary units, and as percent of control values which were always determined simultaneously.*

RESULTS

Anticonvulsant effect

Table 1 presents the anticonvulsant effects of NSD 2023, five known MAO inhibitors, and two clinically used antiepileptic drugs, measured 30 min after subcutaneous administration, and 120 min after oral administration. After subcutaneous

TABLE 1. MEAN PROTECTING DOSES (ED_{50}) AGAINST MAXIMAL ELECTROSHOCK SEIZURES (M.E.S.) AND RESERPINE-PTOSIS. MEAN LETHAL DOSES (LD_{50}). MICE

	ED_{50} mg/kg			LD_{50} mg/kg	
	M.E.S.				
Substance	30 min after s.c. adm.	120 min after p.o. adm.	Reserpine* ptosis p.o. adm.	s.c.	p.o.
NSD 2023	20	>200	20	700	800
Iproniazid	200	>200	20	1000	1000
Isocarboxazide	160	>200	20	700	880
Phenelzine	>50	>50	5	150	130
Tranlylcypromine	>10	>10	?	30	20
Pargyline	>100	>100	35	380	680
Phenytoin	18	20	—	400	1000
Phenobarbital	30	14	—	300	350

* The test compounds were administered orally 3 times in 24 hr. Reserpine (1·0 mg/kg i.v.) was given 3 hr after the last dose.

administration NSD 2023, phenytoin and phenobarbital gave good protective effects, while iproniazid and isocarboxazide had weaker effects. The other substances tested had no protective effect at the dose levels used. When the same substances were administered orally, and the mice tested 2 hr later, only phenytoin and phenobarbital protected against the seizures. The last two substances exhibited good protective effect even 24 hr after administration.

That the route of administration is important in producing the anticonvulsant effect was confirmed by another experiment (Table 2) in which one group of mice were given NSD 2023 (100 mg/kg) orally while another group received the same dose

* Arvid Carlsson, *Pharmac. Rev.* **11**, 490, (1959), reports the concentration of NA in whole rat brain to be 0·49 μg/g. Snyder *et al.*, *Biochem. Pharmac.* **14**, 831, (1965), using two different methods, report the concentration of 5-HT in whole rat brain to be 0·44 and 0·45 μg/g.

subcutaneously. After subcutaneous administration complete protection was obtained for at least 30 min, while after oral administration only partial protection was observed at 5 min and none at 30 min.

TABLE 2. PROTECTION AGAINST ELECTROSHOCK SEIZURES IN MICE AFTER SUBCUTANEOUS AND ORAL ADMINISTRATION OF NSD 2023

	Number of mice out of 6 showing convulsions				
Interval from adm. to shock min	5	15	30	60	120
NSD 2023 100 mg/kg s.c.	0	0	0	4	5
NSD 2023 100 mg/kg p.o.	2	5	6		

Acute toxicity in mice

Table 1 also gives the acute toxicity of NSD 2023, five of the known MAO inhibitors, phenytoin and phenobarbital in mice after oral or s.c. administration. Mice treated with high doses of NSD 2023 showed a short (30 to 60 min) tranquilization and then became normal in appearance. High doses of the known MAO inhibitors increased spontaneous activity and excitability.

Antagonism of reserpine-ptosis

Reserpine-ptosis is prevented by pretreating mice with NSD 2023 (Table 1). Iproniazid and isocarboxazide showed the same activity, phenelzine and tranylcypromine had a stronger effect and pargyline a weaker effect. Varying the time interval between NSD 2023 and reserpine, it can be seen (Table 3) that after a single oral dose of NSD 2023, protection against ptosis is obtained with intervals up to 4 hr.

TABLE 3. ANTAGONISM OF RESERPINE INDUCED PTOSIS

Treatment	Interval from NSD 2023 to reserpine (hr)	Number of mice out of 6 with ptosis after reserpine	
		3 hr	24 hr
Reserpine 1·0 mg/kg i.v.	—	6	6
NSD 2023 50 mg/kg p.o. + Reserpine 1·0 mg/kg i.v.	0·5	1	3
	1·0	1	1
	2·0	1	1
	4·0	0	3
	6·0	—	6

Antagonism of reserpine-hypothermia

NSD 2023 was found to be effective in preventing reserpine induced hypothermia, another symptom in the reserpine syndrom (Table 4). Pretreatment of mice with 3×50 mg/kg orally completely prevented reserpine hypothermia. The ED_{50} for NSD 2023, tested in this way, was about 3×12 mg/kg. Iproniazid showed similar protection, but imipramine had a weaker effect when the temperature was measured 21 hr after reserpine.

TABLE 4. ANTAGONISM OF RESERPINE INDUCED HYPOTHERMIA

Pretreatment orally	Reserpine mg/kg i.v.	Body temperature (°C ± S.E.M.) after reserpine			
		0 hr	2 hr	4 hr	21 hr
NSD 2023	1·0	36·9 ± 0·3	35·1 ± 0·9	34·9 ± 1·0	36·1 ± 1·5
Iproniazid	1·0	37·6 ± 1·0	36·1 ± 0·7	35·5 ± 1·2	36·1 ± 1·8
Imipramine	1·0	36·7 ± 0·3	36·2 ± 0·3	35·2 ± 0·8	32·0 ± 1·1
	1·0	37·1 ± 0·4	35·5 ± 0·8	32·8 ± 1·0	23·4 ± 0·7
	0	37·1 ± 0·5	37·1 ± 0·5	36·9 ± 1·0	36·0 ± 0·6

The test substances (dose 50 mg/kg) were administered (6 mice/dose) 3 times in 24 hr. Reserpine was administered 3 hr after the last treatment.

Effect of NSD 2023 on the cerebral levels of noradrenaline and serotonin in the rat

A single oral dose (50 mg/kg) of NSD 2023 produces a moderate increase in the concentration of serotonin in rat brain, and a smaller, but probably not significant increase in noradrenaline (Table 5). This elevation in the concentration of brain serotonin is of relatively short duration, disappearing completely within 24 hr. In contrast, phenelzine (50 mg/kg) and tranylcypromine (5 mg/kg) produce larger increases in the cerebral concentrations of both serotonin and noradrenaline, which are still quite pronounced 48 hr after administration.

TABLE 5. INCREASE IN RAT BRAIN NA AND 5-HT AFTER ADMINISTRATION OF NSD 2023, PHENELZINE OR TRANYLCYPROMINE

Oral treatment	4 hr		24 hr		48 hr	
	NA	5-HT	NA	5-HT	NA	5-HT
NSD 2023	44	48	29	36	35	26
50 mg/kg	56	48	30	36	37	26
	(103)	(146)	(80)	(96)	(94)	(84)
Phenelzine	70	64	66	84	61	50
50 mg/kg	60	71	65	87	59	49
	(134)	(204)	(178)	(228)	(156)	(160)
Tranylcypromine	66	64	50	49	55	44
5 mg/kg	72	63	56	52	58	46
	(142)	(193)	(144)	(135)	(148)	(145)
None	52	34	36	39	34	30
	45	32	38	36	43	32
	(100)	(100)	(100)	(100)	(100)	(100)

Two rats were used per treatment. The values for each rat are given in arbitrary units of noradrenaline and serotonin per brain. The numbers in parentheses are the averages expressed as per cent of control.

NSD 2023 shows a more pronounced effect in antagonizing the reserpine induced depletion of brain amines. This effect of NSD 2023 is maximum when the interval between NSD 2023 and reserpine is 4 hr or less (Table 6). When the interval is increased to 24 hr no anti-reserpine activity is observed. Under the same conditions iproniazid prevents the reserpine induced depletion of brain amines using intervals of both 3 hr and 24 hr.

In another set of experiments the interval between NSD 2023 and reserpine was held constant, and the brain amines were assayed at different times after reserpine. A typical experiment is presented in Table 7. Rats are pretreated 3 times with 50 mg/kg NSD 2023. Reserpine (1 mg/kg i.v.) is administered 3 hr later, and the rats are killed

TABLE 6. ANTAGONISM BY NSD 2023 AND IPRONIAZID OF RESERPINE INDUCED DEPLETION OF BRAIN AMINES: EFFECT OF VARYING INTERVAL BETWEEN NSD 2023 AND RESERPINE

Pretreatment	Interval from pretreatment to reserpine (hr)	Reserpine mg/kg i.v.	NA	5-HT
0	—	0	41	61
			47	67
			(100)	(100)
0	—	2·5	8	18
			11	19
			(22)	(29)
NSD 2023	3	2·5	41	72
			36	77
			(87)	(116)
NSD 2023	24	2·5	5	6
			5	8
			(11)	(11)
Iproniazid	3	2·5	52	133
			37	126
			(103)	(202)
Iproniazid	24	2·5	74	103
			57	96
			(145)	(156)

NSD 2023 and Iproniazid (50 mg/kg) were given orally three times in 24 hr. Three and 24 hr after the last administration reserpine was given (2·5 mg/kg, i.v.). The rats were killed 2 hr after the reserpine injection. Two rats were used per treatment. The numbers represent arbitrary units of NA and 5-HT per brain. The numbers in parentheses are averages presented as per cent of the control.

TABLE 7. ANTAGONISM BY NSD 2023 OF THE DEPLETION OF RAT BRAIN NORADRENALINE AND SEROTONIN INDUCED BY RESERPINE

	Pretreat with NSD 2023	Reserpine 3 hr later	Kill 3 hr after reserpine or 6 hr after NSD 2023		Kill 24 hr after reserpine or 27 hr after NSD 2023	
	mg/kg	mg/kg	5-HT	NA	5-HT	NA
(1)	0	0	30	88	34	104
			26	99	32	98
			(100)	(100)	(100)	(100)
(2)	0	1·0	8	14	16	14
			8	16	12	14
			(30)	(16)	(44)	(14)
(3)	3 × 50 mg/kg	0	40	116	34	100
			42	122	34	98
			(145)	(128)	(102)	(98)
(4)	3 × 50 mg/kg	1·0	18	31	26*	60†
			18	40	22	52
			(64)	(38)	(74)	(56)

Rats were pretreated with NSD 2023 (50 mg/kg orally) 3 times in 24 hr. Reserpine (1 mg/kg, i.v.) was administered 3 hr after the last dose of NSD 2023. The rats were killed 3 or 24 hr after reserpine, and 6 or 27 hr after NSD 2023. Two rats were used per treatment. The assays for 5-HT and NA are given for each rat brain (in arbitrary units). The numbers in parentheses are averages expressed as percent of control.

* Differs from control group with $P_{14} = 0{\cdot}06$, and from group receiving reserpine alone with $P_{24} = 0{\cdot}08$.

† $P_{14} = 0{\cdot}02$, and $P_{24} = 0{\cdot}01$.

3 hr and 24 hr after reserpine. It can be seen that in rats treated first with NSD 2023 then with reserpine, the amounts of brain 5-HT and NA are significantly greater than in rats treated with reserpine alone, and significantly smaller than control rats or rats treated with NSD 2023 alone. These differences are observable at both 3 and 24 hr after reserpine.

In vivo *inhibition of mouse brain MAO*

The increase in brain 5-HT produced by NSD 2023 suggested that this substance might be an inhibitor of MAO *in vivo*. A series of experiments were therefore performed to determine the effect of NSD 2023 on mouse brain MAO *in vivo*, using tryptamine and kynuramine as substrates.

Fig. 2 shows the MAO activity in whole homogenates of brains taken from mice treated 1 hour previously with single oral doses of various amounts of NSD 2023. It can be seen that 100 per cent inhibition is not approached with the doses used. Using tryptamine as substrate it appears that a limiting value of about 70 per cent inhibition is approached: at 200 mg/kg MAO inhibition is not significantly different from that obtained with 20 mg/kg.

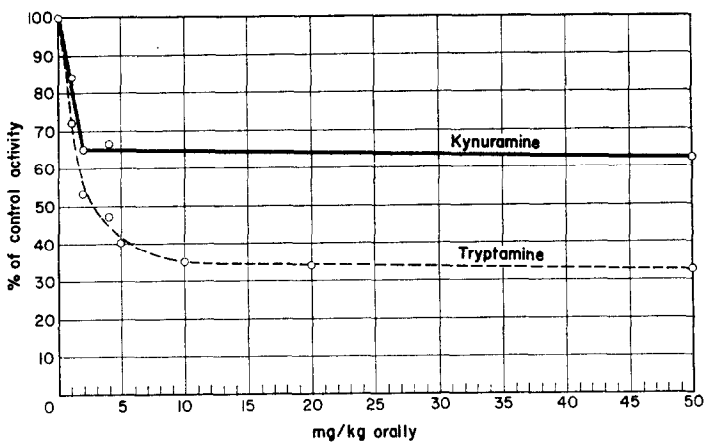


FIG. 2. The inhibition of mouse brain MAO *in vivo* by NSD 2023 as a function of dose. NSD 2023 was administered orally and the mice were killed 1 hr later. Two mice were used per treatment. In the experiment using kynuramine as substrate, the usual homogenates were diluted 20 fold in potassium phosphate 50 mM pH 7·4 containing 1 mM EDTA, then assayed as described under Materials and Methods. The average difference between two mice receiving the same treatment was about 13 per cent of the mean.

Using kynuramine as substrate, maximum inhibition was about 40 per cent. With either tryptamine or kynuramine as substrate, the dose of NSD 2023 required to produce 50 per cent of maximum inhibition was between 1 and 2 mg/kg. These results were consistently obtained in several separate experiments.

The duration of MAO inhibition *in vivo* was found to be dose dependent (Fig. 3). The time required for the regeneration of 50 per cent of inhibited enzyme ranged from 5 to 17 hr, after doses of 2 and 200 mg/kg, respectively. In contrast, the 50 per cent regeneration times (RT_{50}) after inhibition by tranylcypromine and iproniazid were

58 hr and 270 hr, respectively. It is of some interest that the rate of regeneration of enzyme activity after "irreversible" inhibition varies considerably with the inhibitor used (Table 8).

TABLE 8. VELOCITY OF ONSET, DURATION AND MAXIMUM MAGNITUDE OF MAO INHIBITION PRODUCED BY NSD 2023 AND FIVE OTHER MAO INHIBITORS, IN MOUSE BRAIN

Substance (dose in mg/kg)		Onset time 50 (min)	Regeneration time 50 (hr)	Maximum inhibition observed (%)
NSD 2023	2	<5 min	5	60
	50	< 5 —	7	80
	200	< 5 —	17	75
Iproniazid	200	60 —	270	89
Isocarboxazide	200	<5 —	240	100
Pargyline	100	<5 —	160	97
Phenelzine	50	<5 —	190	98
Tranylcyrpomine	5	10 —	58	98

The 50 per cent onset times (OT_{50}) are the times required, after oral administration, for 50 per cent of maximum inhibition to occur.

The 50 per cent regeneration times (RT_{50}) are the times required, after oral administration, for 50 per cent of inhibited enzyme to be regenerated.

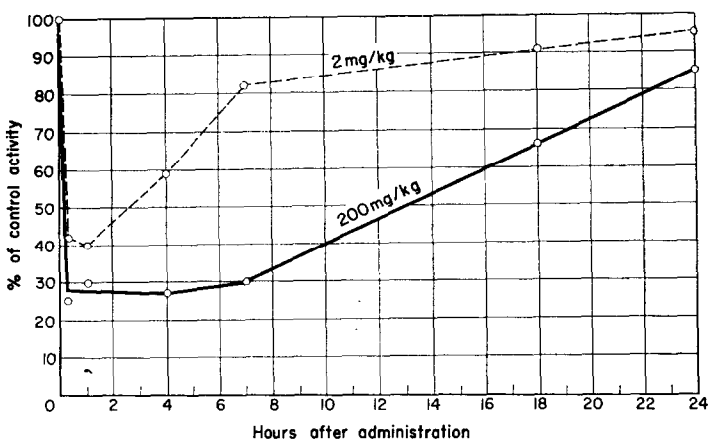


FIG. 3. The inhibition of mouse brain MAO by NSD 2023 as a function of time after a single oral dose (2, or 200 mg/kg). Tryptamine was used as substrate.

Repeated administrations of NSD 2023 neither significantly prolong inhibition, nor increase it above the limiting value observed after a single administration (Table 9).

In vitro *inhibition of mouse brain MAO*

In vitro inhibition of mouse brain MAO by NSD 2023 is similar to that produced *in vivo* in that only partial inhibition is oberved. However, the maximum inhibition produced *in vitro* is less than that produced *in vivo*. Furthermore, the apparent concentration of NSD 2023 required to produce half-maximum inhibition *in vivo* is much less than the concentration required to give half-maximum inhibition *in vitro*.

TABLE 9. EFFECT OF REPEATED ORAL ADMINISTRATION OF NSD 2023 ON INHIBITION OF MOUSE BRAIN MAO

NSD 2023 Orally	Interval between last administration and killing	% of control
20 mg/kg 5 times*	1 hr	36
20 mg/kg 5 times*	25 hr	86
50 mg/kg 4 times†	30 min	28

* NSD 2023 was administered orally 2 times a day (at 8.00 and 15.30) for a total of 5 times.

† NSD 2023 was administered orally 4 times at 1 hr intervals.

This is in striking contrast to many of the known irreversible MAO inhibitors which are much more effective *in vitro* than *in vivo* (comparing mg/kg with μg/ml).[14]

Fig. 4 shows that with tryptamine as substrate maximum inhibition is about 50 per cent and the concentration of NSD 2023 required to produce 50 per cent of maximum inhibition is approximately 20 μg/ml.

When kynuramine is used as substrate, however, an entirely different result is obtained; 100 per cent inhibition is approached with increasing NSD 2023 concentration. At pH 7·8 half maximum inhibition is obtained with a concentration of 145 μg/ml.

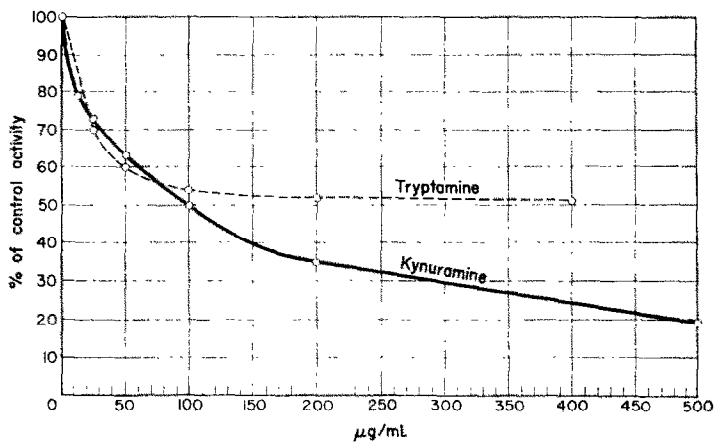


FIG. 4. Inhibition of mouse brain MAO *in vitro* by NSD 2023, using tryptamine and kynuramine as substrates. With kynuramine as substrate the brain homogenate was diluted 20-fold in buffer pH 7·8, containing Tris HCl 100 mM, $NAPO_4$ 100 mM, and EDTA 1 mM. 200 μl homogenate and 100 μl inhibitor were pre-incubated together for 20 min.

The apparently greater inhibitory activity of NSD 2023 *in vivo* suggested that *in vivo* inhibition might be due to a metabolite of NSD 2023. Dubnick *et al.*[15] have shown that 2-methyl-3-piperidinopyrazine, a weak MAO inhibitor *in vitro*, is converted *in vivo* into a much more potent inhibitor. In mice this conversion is blocked by pretreatment with SKF 525A. However, several experiments failed to show any effect of pretreatment with SKF 525A on MAO inhibition produced by subsequent administration of NSD 2023 (Table 10).

TABLE 10. EFFECT OF PRETREATMENT WITH SKF 525A ON INHIBITION OF MOUSE BRAIN MAO BY NSD 2023 *in vivo*

	NSD 2023 mg/kg orally	Pretreatment with SKF 525A	
		0	20 mg/kg
A	0	62	64
		63	71
	5	20	25
		23	21
	10	19	19
		20	25
		0	50 mg/kg
B	0	47	42
		48	43
	2	34	32
		34	36

In experiments A and B mice were pretreated orally with SKF 525A, 20 and 50 mg/kg, respectively, 30 min before NSD 2023. Mice were killed 15 min after NSD 2023, and brain MAO determined using tryptamine as substrate. The results are given in arbitrary units of MAO per brain.

Protection of MAO in vivo

It has been shown that harmine or harmaline pretreatment of rats and mice can protect brain MAO from irreversible inhibition by other MAO inhibitors.[15–17] Since the duration of inhibition produced by NSD 2023 is much shorter than that produced by phenelzine, tranylcypromine, iproniazid, and pargyline, NSD 2023 was tested for its ability to prevent inhibition by these substances. The results are given in Table 11. It is seen that the irreversible inhibition produced by the four inhibitors, measured 18 hr after oral administration, is significantly reduced by pretreatment with a single oral dose (50 mg/kg) of NSD 2023.

In another type of experiment harmaline was tested for its ability to protect MAO from inhibition by NSD 2023. Preliminary experiments showed that MAO inhibition produced by NSD 2023 was only slightly reversed by repeated washing of the brain mitochondria, while this treatment reduced inhibition due to harmaline to 10 per cent or less. It is therefore possible to remove the effect of harmaline while retaining that due to NSD 2023. In Table 12 it can be seen that pretreatment of mice with harmaline significantly reduced the inhibition of MAO due to subsequent administration of NSD 2023.

These results suggest that both substrate and the inhibitors examined here are bound by the same site on MAO. The possibility is not excluded, however, that substrate and inhibitor (or two inhibitors) may be bound by separate, but strongly interacting binding sites, an effect similar to the well-known substrate protection of many enzymes against non-specific irreversible inhibition.

Organ specificity

Organ specificity has been reported for several MAO inhibitors.[52, 53] Table 13 shows that NSD 2023 also exhibits organ selectivity, inhibiting MAO to a greater extent in brain and kidney, than in liver, both *in vivo* and *in vitro*. Inhibition *in vivo*, which is observed in all three organs at 15 and 60 min after administration has completely disappeared at 24 hr.

TABLE 11. THE EFFECT OF PRETREATMENT WITH NSD 2023 ON THE INHIBITION OF MOUSE BRAIN MAO BY IRREVERSIBLE INHIBITORS

	Pretreatment with NSD 2023	Treatment with irreversible MAO inhibitor	MAO units/brain	Average	Per cent of control	P
A	0	0	62			
			57	60	100	
	+	0	54			
			53	54	90	
	0	Phenelzine	2			
		50 mg/kg	3	2	3	0·04
	+	Phenelzine	23			
		50 mg/kg	33	28	47	
	0	Tranylcypromine	13			
		5 mg/kg	13	13	22	0·001
	+	Tranylcypromine	47			
		5 mg/kg	45	46	73	
B	0	0	68			
			64	66	100	
	+	0	61			
			56	59	89	
	0	Iproniazid	3			
		200 mg/kg	3	3	5	0·008
	+	Iproniazid	17			
		200 mg/kg	15	16	24	
	0	Pargyline	5			
		200 mg/kg	6	6	9	0·001
	+	Pargyline	28			
		200 mg/kg	29	28	43	

Mice were pretreated with NSD 2023 50 mg/kg orally. Thirty minutes later the irreversible MAO inhibitor was administered orally. The mice were killed 18½ hr after administration of NSD 2023 and 18 hr after irreversible inhibitors. MAO was determined by the method of Lovenberg *et al.* using tryptamine as substrate.

TABLE 12. EFFECT OF PRETREATMENT WITH HARMALINE ON THE INHIBITION OF MOUSE BRAIN MAO BY NSD 2023 *in vivo*

Pretreatment	Second treatment	MAO units/brain	Average	Per cent of control	
None	none	61			
		66	64	100	
Harmaline	none	56			
		58	57	90	
None	NSD 2023	27			
		25	26	41	P = 0·004
Harmaline	NSD 2023	51			
		49	50	79	

Mice were pretreated with harmaline 100 mg/kg orally. Thirty minutes later NSD 2023 was administered, 50 mg/kg orally. The mice were killed 1 hr after harmaline, or 30 min after NSD 2023. Each brain was homogenized in 2 × 5·0 ml sucrose 0·25 M containing 1 mM EDTA. The homogenate was centrifuged for 10 min at 18,000 *g*. The resulting supernatant was discarded, and the pellet was resuspended in 10 ml of sucrose-EDTA, and allowed to stand overnight at approx. 4°, and centrifuged again (10 min at 18,000 *g*.). The pellet was taken up in 5·0 ml of EDTA-phosphate, and MAO determined on a 200 µl aliquot using tryptamine as substrate.

Species specificity

Using an oral dose of 50 mg/kg the inhibition of MAO *in vivo* in the brains of guinea pigs and rats was found to be 45 and 76 per cent respectively. The RT_{50}'s were found to be 3·3 and 10 hr, respectively.

TABLE 13. INHIBITION OF MAO IN MOUSE BRAIN, KIDNEY, AND LIVER BY NSD 2023

Organ	Percent of control MAO after 50 mg/kg NSD 2023		
	kill 15 min	60 min	24 hr
(a) *in vivo*:			
Brain	40	36	100
Kidney	40	36	109
Liver	77	71	113
(b) *in vitro*:			
Organ	Percent of control MAO with 50 μg/ml NSD 2023		
Brain		54	
Kidney		49	
Liver		82	

Brain, 2 kidneys, and liver from mouse were homogenized in 2 × 1·5 ml, 2 × 1·0 ml, and 2 × 5·0 ml, respectively, of EDTA-PO_4. MAO activity was determined on a 100 μl aliquot using tryptamine as substrate. The relative MAO activities by this method are:
Brain = 100, 2 kidneys = 31, liver = 370.

In vitro, rat brain MAO was inhibited 38 per cent by NSD 2023 at a concentration of 1 mg/ml. Guinea pig brain MAO was inhibited 9 per cent at 400 γ/ml.

DISCUSSION

Since NSD 2023 exhibits both antireserpine and MAO inhibitory activities, it is of some interest to consider whether the two effects are causally related.

The antireserpine and MAO inhibitory effects of NSD 2023 are of comparable duration. On the other hand, the ED_{50} values for the two effects are 20 and 1·4 mg/kg, respectively, i.e. MAO is almost maximally inhibited by doses of NSD 2023 which produce little antireserpine effect, a finding which suggests the lack of a causal relationship between antireserpine activity and MAO inhibition as measured using tryptamine or kynuramin as substrates. This apparent discrepancy might be resolved in one of two ways: (1) it is conceivable that, if 5-HT or NE had been used as substrates, instead of tryptamine and kynuramine, the correspondence between the two effects might have been closer. (2) A non-linear relationship between MAO inhibition and antireserpine activity may exist. The second possibility receives support from the work of Gey and Pletscher[18] who found that, using iproniazid, MAO in rat brain must be inhibited at least 85 per cent before a significant increase in cerebral 5-HT or NE can be measured. This effect was attributed to the existence of excess MAO, although it seems also possible that a better correlation between increase in cerebral amine levels and MAO inhibition might have been obtained if NE or 5-HT had been used as substrates in their MAO determinations.

It has been proposed that the antireserpine effect of the MAO inhibitors might be due to the increased levels of one or another of the biogenic amines which could then

more effectively compete with reserpine in the initial (reversible) phase of its binding to the storage granules.[19, 20, 51] However, it seems improbable that NSD 2023 produces its antireserpine effect by this mechanism since a single dose, sufficient to antagonize reserpine, produces a relatively small increase in the cerebral concentration of 5-HT and no significant elevation of brain NA. It appears, on the other hand, that NSD 2023 may, by direct action, prevent reserpine from reaching the binding sites at which it produces its effects. A similar, although weaker, action has been reported for desmethylimipramine.[22, 23]

Although reserpine antagonism appears to be a general property of MAO inhibitors, the mechanism(s) by which these effects are produced is not clear. This is due, first of all, to the uncertainty as to the mechanism by which reserpine produces its pharmacological effects. Early work with reserpine suggested that the syndrome produced by this substance might be causally related to overall depletion of one or another of the biogenic amines.[24, 25] This conclusion, however, was not supported by subsequent work.[26–30] Recently it has been shown that the reserpine syndrome is better correlated with the ability of tissues to store amines than with overall amine content.[31, 32] Some evidence also exists that reserpine, by direct action, may: (1) block adrenergic receptors[33] (2) inhibit the transport of NA across the nerve cell membrane,[34] and inhibit the synthesis of NA.[36]

The picture is further complicated by observations which suggest that some of the pharmacological effects of the MAO inhibitors may be, at least partly, produced by mechanisms other than MAO inhibition. These include direct effects on the synthesis, storage and release of amines,[35–40] as well as depression of ganglionic and neuromuscular transmission (ref. 2, pp. 498–9).

Taken together, these findings cast doubt on the idea that the antireserpine action of MAO inhibitors is solely due to MAO inhibition.

However, even if MAO inhibition and antireserpine activity are not causally related, it is probably not a coincidence that the two effects are often produced by the same substances: if the MAO inhibitors have a strong affinity for the amine binding sites on MAO, as is suggested by the protection experiments, it would seem reasonable to assume that they may also exhibit affinity for amine binding sites of other types, e.g. (1) post synaptic receptors (2) binding sites in the storage granules (3) transport sites on the cell membrane (4) feed-back inhibition sites, probably located on the tyrosine and tryptophan hydroxylases, which are thought to catalyse the rate limiting reactions in the formation of NA[41] and 5-HT[42] in the brain.

Similarly, reserpine and related substances may be expected to exhibit affinity for amine binding sites other than those in the storage granules. MAO inhibitors and reserpine, therefore, may compete directly for common binding sites of several types.

The anticonvulsant effect of NSD 2023 appears to be clearly independent of MAO inhibition as measured using tryptamine or kynuramine as substrates: neither the ED_{50}'s nor the durations of the two effects correspond. Furthermore, a significant anticonvulsant effect is observed after s.c. but not after oral, administration of NSD 2023, while MAO inhibition is not dependent on route of administration. Iproniazid and isocarboxazid also exhibited a weak anticonvulsant effect, which is, in all probability, not related to MAO inhibition (ref. 2, p. 380).

It is of interest that although the ability of reserpine to antagonize the anticonvulsant activity of diphenylhydantoin can be prevented by pretreatment with MAO inhibitors,

this action of reserpine does not appear to be related to the overall depletion of amines in the brain.[27]

It was shown that NSD 2023 is a more effective inhibitor of mouse brain MAO *in vivo* than *in vitro*, using tryptamine as the substrate. This finding suggests that inhibition *in vivo* may be primarily due to a metabolite of NSD 2023. However, pretreatment of mice with SKF 525A had no measurable effect on MAO inhibition produced by subsequent administration of NSD 2023. It may therefore be concluded that *in vivo* mouse brain MAO is inhibited by NSD 2023 as such in conjunction with factors at present unknown, or by a metabolite of NSD 2023 the formation of which is not blocked by SKF 525A.

The finding that mouse brain MAO is only partially inhibited by NSD 2023, both *in vivo* and *in vitro*, using tryptamine as substrate, suggests that tryptamine is deaminated by two or more separate enzymes not all of which are inhibited by NSD 2023. The observation that NSD 2023, inhibits liver MAO less than brain MAO may also be explained by assuming that the NSD 2023 sensitive MAO constitutes a smaller fraction of total MAO activitity in liver than it does in brain.

The existence of multiple forms of MAO, having different sensitivities to certain MAO inhibitors, is indicated by the finding that, using a given inhibitor, the concentsation required to produce 50 per cent inhibition, depends dramatically on the substrates used.[43–45] Another example of this phenomenon was recently described by Huszti an Borsy[46] who showed that the deamination of mescaline by rabbit liver mitochondria was strongly inhibited by the substance T-134 at concentrations which had no measurable effect on the deamination of 3,4-dimethoxyphenethylamine, and tyramine. It is of interest that T-134 is structurally similar to NSD 2023.

Other evidence is consistent with the assumption that different forms of MAO also differ with respect to pH optimum,[47, 48] thermo-stability,[21, 50] and other physical properties.[49]

The problem of relating pharmacological activity to MAO inhibition is considerably complicated by the existence of multiple forms of MAO, since a given pharmacological effect might depend on the specific inhibition of only one of the forms.

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