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ANTIHYPERTENSIVE AND MONOAMINE OXIDASE INHIBITORY ACTIVITY OF 3-AMINO-2-OXAZOLIDINONE (3AO) AND ITS CONDENSATION PRODUCT WITH 2-SUBSTITUTED-3-FORMYL-4-OXO-(4H) PYRIDO(1,2-a)PYRIMIDINES*

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Abstract—Some pharmacological and biochemical properties of 3-amino-2-oxazolidinone (3AO) and its condensation products with 2-substituted-3-formyl-4-oxo(4H)-pyrido(1,2-a)pyrimidines are described. All these compounds showed powerful antihypertensive and monoamine oxidase (MAO) inhibitory properties. In some cases both these activities were two to four times those of pargyline. Although these compounds were powerful MAO inhibitors *in vivo*, they were inactive *in vitro*. Pretreatment with SKF525A failed to show any significant effect on the MAO inhibition produced by these compounds (except Compound A). The correlation between the antihypertensive and MAO inhibition was not very good.

WE HAVE previously reported the antihypertensive and monoamine oxidase (MAO) inhibitory properties of two series of compounds, (a) some azacycloalkyl substituted benzaldehyde-hydrazone derivatives[1] (b) some derivatives of 3-formyl-4-oxo(4H)-pyrido(1,2-a)pyrimidine.[2] Most of the compounds of these series showed good antihypertensive and MAO inhibiting properties *in vivo*. The MAO inhibiting activity was more towards liver than brain MAO. Despite the fact that some of these compounds were more potent than pargyline *in vivo* as regards MAO inhibition, they were practically inactive *in vitro*. Many of the compounds in these two series had 3-amino-2-oxazolidinone (3AO) as a part of the molecule and compounds synthesized without this residue showed no significant antihypertensive and MAO inhibiting activity.

(3AO) by itself exhibited a powerful antihypertensive effect in renal hypertensive rats and inhibited brain and liver MAO *in vivo* but not *in vitro*. In this respect 3AO resembled some of these compounds which are shown in Fig. 1, which were synthesized by the condensation of 3AO with 2-substituted-3-formyl-4-oxo(4H)pyrido (1,2-a)-pyrimidines. Since 3AO was a common denominator in all the compounds of this series, it was therefore of interest to see if their antihypertensive and MAO inhibitory effects were similar to those seen with 3AO. It was interesting to note that like 3AO these compounds were active in inhibiting MAO only *in vivo* and not *in vitro*. Further a comparison of these compounds with other known MAO inhibitors, namely pargyline and tranylcypromine has been made.

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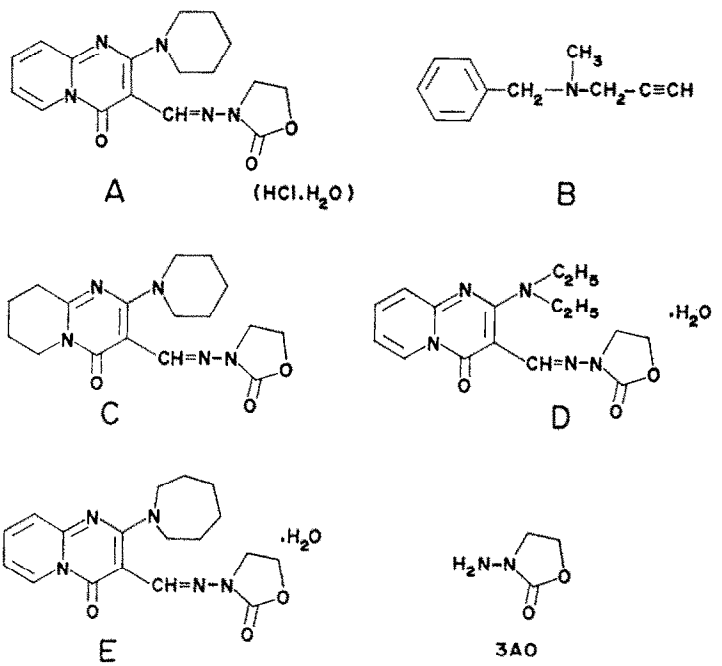

FIG. 1. Structural formulae of compounds A, B (pargyline), C, D, E and 3-amino-2-oxazolidinone (3AO).

METHODS

(a) *Effect on blood pressure.* Cats and dogs of either sex were anaesthetized with pentobarbitone, 45 mg/kg i.p. and 35 mg/kg i.v. respectively. Blood pressure was recorded from a carotid artery. In some experiments the blood pressure was recorded on a 7 channel grass polygraph by means of a pressure transducer ($P_{23}Dc$). The heart rate was monitored by means of E.C.G. taken from lead II. All drugs were given intravenously.

In a second group of experiments the effects of these compounds on the hypertension induced by stimulation of peripheral sympathetic ganglia by DMPP was studied in adrenalectomized cats.

(b) *Tyramine potentiation.* Series of experiments were done in which control responses to different doses of tyramine on blood pressure were taken in cats, dogs and rats. Different doses of tyramine were given at an interval of 30–45 min to avoid tachyphylaxis. Cats, dogs and rats were then pretreated with various compounds for 4 days (doses given in figures) and the sensitivity to tyramine checked again. The rats used for these experiments were pithed.

(c) *Renal hypertensive rats.* Renal rats were prepared by bilateral clamping of their renal arteries according to the method of Goldblatt *et al.*[3] They were given 2, 5, 30 and 100 mg/kg (p.o.) of various substances and blood pressure was measured by plethysmographic method from the tail under light ether anaesthesia.[4]

(d) *Catecholamine estimation.* The tissues were extracted with 2% PCA, adsorbed on acid washed alumina at pH 8·4 and eluted with 0·2 N acetic acid as described by

Crout *et al.*[5] Dopamine was estimated as described by Carlsson and Waldeck with some modifications.[6,7] The endogenous 5HT content of the brain was measured, according to the method of Anden and Magnusson.[8]

(e) *Monoamine oxidase inhibition.* MAO inhibition in the rat brain and liver homogenates was measured using two different substrates, namely, kynuramine[9,10] and 5-hydroxytryptamine.[8] In the case of kynuramine, the appearance of 4-hydroxyquinoline was measured, and in the case of 5-hydroxytryptamine its disappearance was measured. All incubations were run for a period of 0·5 hr in the case of kynuramine and 1 hr in the case of 5-hydroxytryptamine. In all *in vitro* studies, preincubation for a period of 0·5–1 hr was carried out before the addition of substrate.

Experiments carried out with SKF525A; rats were injected with SKF525A (50 mg/kg i.p.) and 1 hr later they were given a dose of the compound under study. The rats were killed 6 hr later and the MAO inhibition was measured.

(f) *Noradrenaline uptake.* This was measured in the rat heart as described by Muscholl.[11]

RESULTS

Intravenous administration of 3AO (3 and 9 mg/kg i.v.) produced a transient rise of blood pressure which lasted for about 10 min. There was no significant effect on the heart rate with both the doses. All the rest of the compounds on intravenous administration (9 mg/kg i.v.) did not produce any significant effect on the blood pressure and heart rate. The pressor response to adrenaline and noradrenaline on the blood pressure were potentiated by all the compounds (the effect was more marked in the case of adrenaline than in the case of noradrenaline, Table 1). Although most of these compounds did not produce any fall of blood pressure in acutely anaesthetized animals, they all lowered blood pressure in renal hypertensive rats (Fig. 2). The fall of blood pressure varied from 26 to 60 per cent. The most potent compound was 3AO which lowered the blood pressure by about 60 per cent when given at 30 mg/kg p.o. for 10 days (Fig. 2).

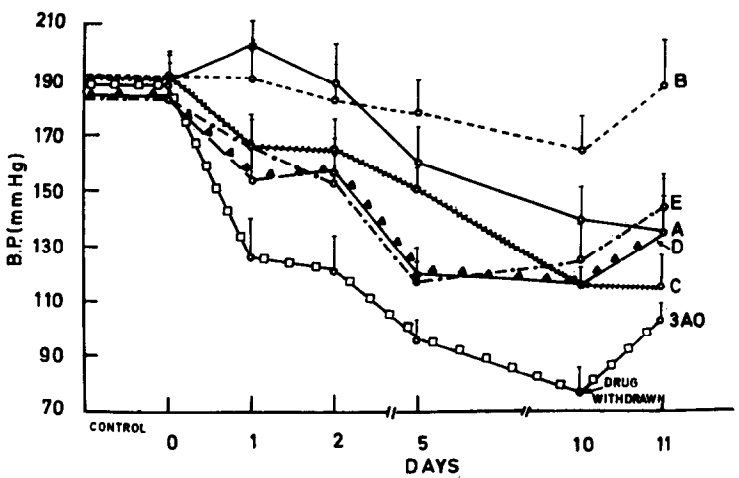

FIG. 2. Effect of compound A, B, C, D and E and 3AO (30 mg/kg p.o. for 10 days) on the blood pressure of renal hypertensive rats. Note the marked antihypertensive response of these compounds as compared to pargyline (B). Each point is a mean of at least six determinations.

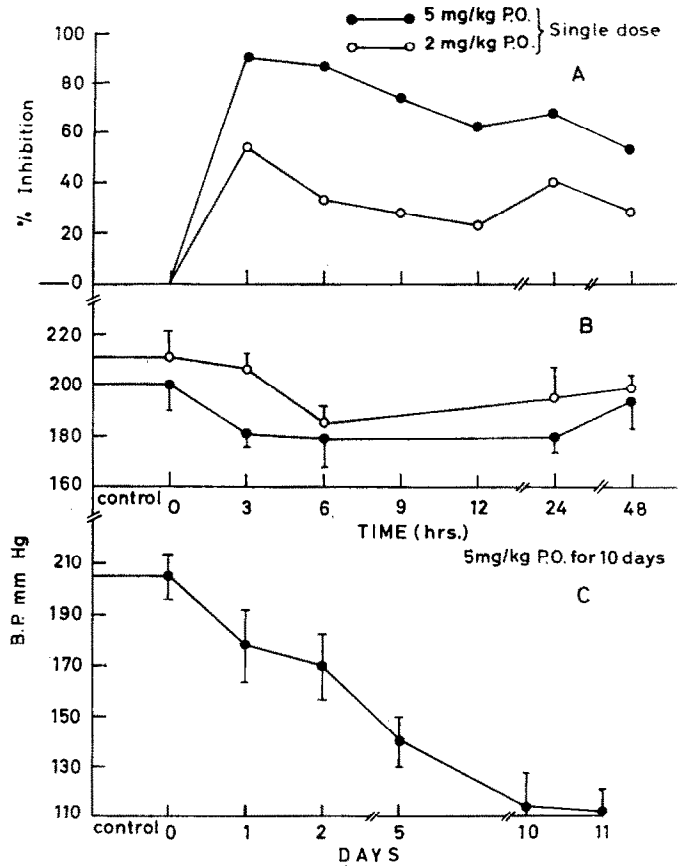

FIG. 3. Correlation between antihypertensive response and MAO inhibition in the rat. A, shows the MAO inhibition produced by 2·5 and 5 mg/kg of 3AO in the rat brain. B, shows the antihypertensive response of similar doses after single dose administration. C, shows the effect of higher dose (5 mg/kg p.o.) given for 10 days. Note that 5 mg/kg p.o. of 3AO (single dose) although producing marked inhibition of MAO does not produce a significant antihypertensive response whereas the same dose repeated for 10 days produces a significant decrease in the blood pressure.

Pargyline was very weak in this respect. It lowered the blood pressure in renal hypertensive rats by 13 and 26 per cent at a dose of 30 and 100 mg/kg p.o. respectively given for 10 days. As compared to pargyline, the antihypertensive activity of these compounds was 2- to 4-fold. Single doses of 3AO, 2 and 5 mg/kg p.o. (doses which produced good inhibitions of liver and brain MAO (see below), was practically ineffective in lowering blood pressure of the rats, when measured up to 48 hr (Fig. 3). However, repeated administration of the same dose produced a significant fall of blood pressure in renal hypertensive rats (Fig. 3).

MAO inhibition in vivo. The effect of these compounds on *in vivo* inhibition of MAO are shown in Figs. 4, 5, 6 and 7. As can be seen from the figures, these compounds produced a dose dependent irreversible inhibition of the enzyme in both liver and brain homogenates using both the substrates. The MAO inhibition was more pronounced in the liver than in the brain. Comparing the MAO inhibitions of 3AO with

TABLE 1. EFFECT OF 3AO (3 mg/kg i.v.) COMPOUND A, C, D AND E (9 mg/kg i.v.) ON THE PRESSOR RESPONSE OF ADRENALINE (4 μg/kg) AND NORADRENALINE (2 μg/kg) IN ANAESTHETIZED CATS

Test substance	% Increase in the pressor response of			
	Adrenaline		Noradrenaline	
	1 hr	2 hr	1 hr	2 hr
3AO	58 (5)	60 (4)	40 (5)	45 (4)
A	29 (6)	24 (6)	No change	No change
C	47 (6)	22 (3)	20 (3)	5 (3)
D	24 (6)	41 (4)	26 (4)	30 (4)
E	32 (6)	52 (3)	No change	13 (3)

Figures in the parenthesis indicate the number of observations.

tranylcypramine, it seems that this substance is more potent than tranylcypramine as regards inhibition of liver MAO and is equipotent as regards inhibition of brain MAO (Fig. 4). The MAO inhibition produced by 3AO was roughly the same when given orally or intravenously (Table 2). Comparison of the *in vivo* MAO inhibitory potency of these compounds with pargyline showed that all the compounds were more potent, except Compound E; the ED_{50} values of various compounds in mg/kg are shown in Table 3.

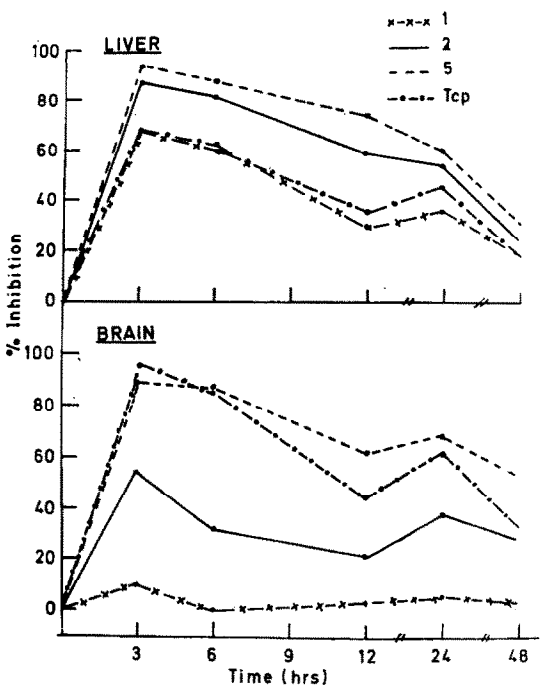

FIG. 4. The inhibition of the rat brain and liver MAO *in vivo* by 3AO and tranylcypromine (TCP), as a function of time after single oral dose (1, 2, 5 mg/kg of 3AO and 5 mg/kg p.o. of TCP). Kynuramine was used as a substrate. Note the graded inhibition produced by 3AO in liver and brain tissues. The inhibition is more towards hepatic than brain MAO. Each point is the mean of at least two determinations.

TABLE 2. COMPARISON OF THE MAO INHIBITORY ACTIVITY OF 3AO WHEN GIVEN ORALLY OR INTRAVENOUSLY

Test compound	Dose (mg/kg)	Route	Inhibition (%) Brain	Liver
3AO	5	i.v.	95 (4)	97 (4)
3AO	5	p.o.	84 (3)	91 (3)
3AO	2	i.v.	73 (2)	89 (2)
3AO	2	p.o.	60 (3)	82 (3)

All animals killed 3 hr after giving the drug. Figures in the parenthesis indicate the number of observations.

TABLE 3. APPROXIMATE DOSES (mg/kg) OF COMPOUNDS A, B (PARGYLINE), C, D AND E REQUIRED TO PRODUCE 50 PER CENT INHIBITION OF MAO *in vivo*

	5HT substrate					Kynuramine substrate				
Organ	A	B	C	D	E	A	B	C	D	E
Brain	27	52	20	17	38	49	45	19	38	82
Liver	15	28	15	15	20	17	20	13	15	18

Inhibitions were calculated for an incubation period of 0·5 hr in the case of kynuramine and 1 hr in the case of 5-hydroxytryptamine. All values are the mean of at least two observations. All animals killed 16 hr after treatment.

The onset of action of all these compounds was quick (although very early time intervals were not studied). Three hr after treatment a significant inhibition of MAO in both tissues was seen (Figs. 4 and 6). The duration of action of these compounds, except 3AO, was very long even after a single administration (100 mg/kg p.o.). Compounds A, B, C, D and E produced a very long lasting inhibition (Fig. 8). Four days after treatment about 50 per cent of the enzyme was still inhibited. Normal values were

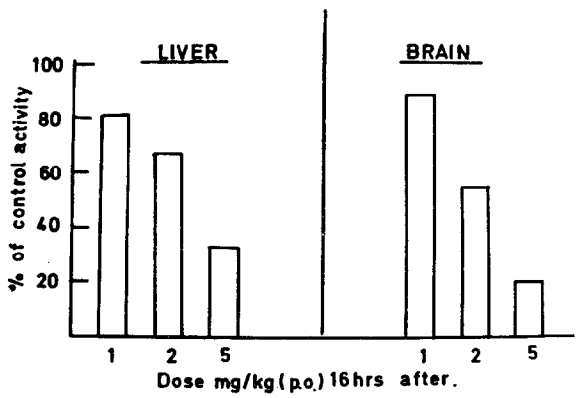

FIG. 5. The inhibition of rat brain and liver MAO by 3AO *in vivo*. 5-Hydroxytryptamine was used as a substrate. Note the graded inhibition produced by 3AO in both liver and brain tissues. Each point is the mean of at least two determinations.

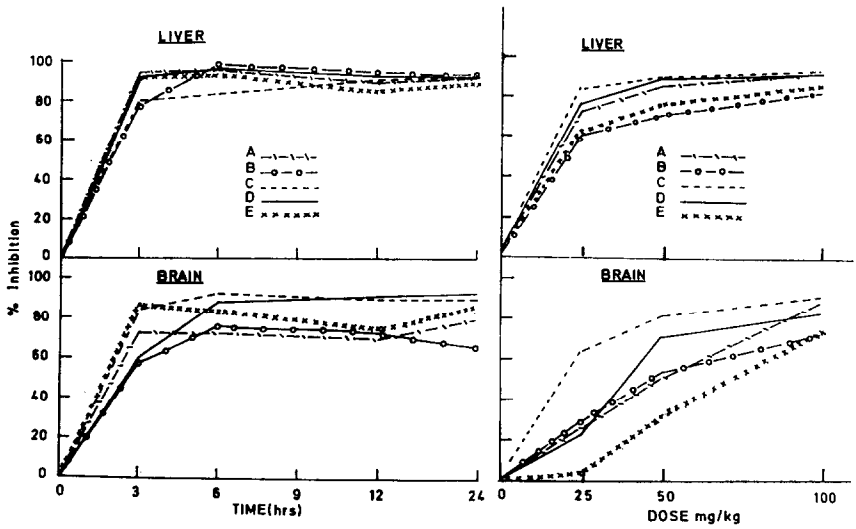

FIG. 6. The inhibition of rat brain and liver MAO by compounds A, B, C, D and E as a function of time (left panel) after 100 mg/kg p.o. and after different dosages (right panel). Note the graded inhibition produced by different doses of the compounds. The liver MAO seems to be inhibited more than the brain MAO. Each point is a mean of at least two determinations.

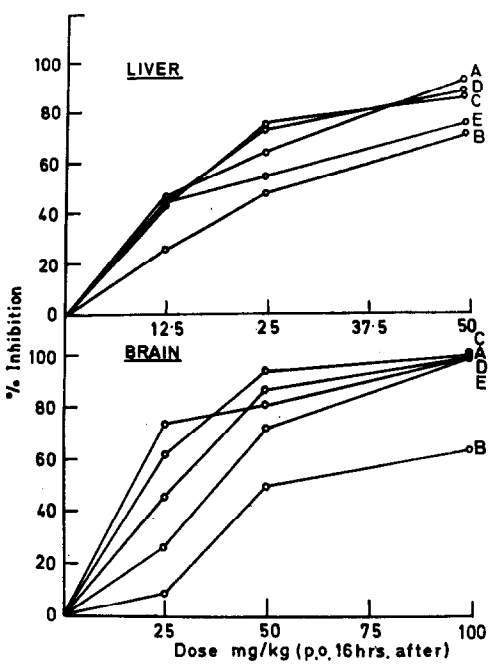

FIG. 7. The inhibition of rat brain and liver MAO *in vivo* by compounds A, B, C, D and E after different doses. 5HT was used as a substrate. Note the marked inhibition of the enzyme in liver at low doses as compared to brain. Each point is a mean of at least two determinations.

seen for all the compounds, except pargyline, within 7 days. Although initially these compounds produced more inhibition of liver than brain MAO, the regeneration time of the enzyme was roughly the same in both tissues (Fig. 8).

MAO inhibition in vitro. All these compounds when added to the brain or liver homogenates up to a dose of 1×10^{-4} produced no inhibition of the enzyme in either tissue, except compound A which produced some inhibition of the enzyme in both

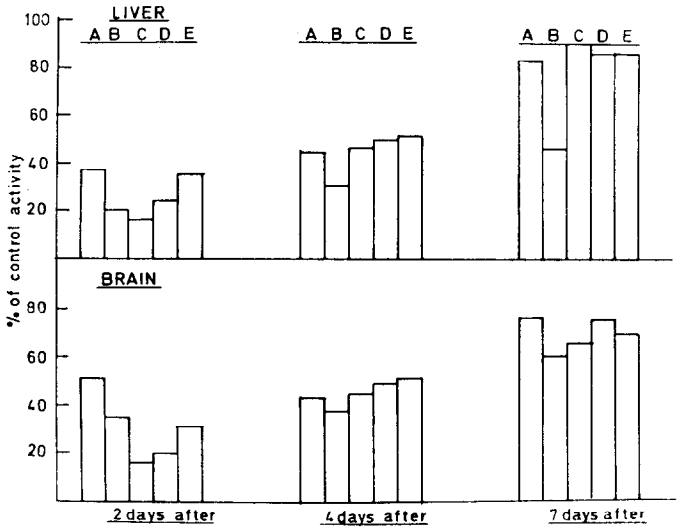

FIG. 8. Duration of the action of compounds A, B, C, D and E on MAO activity (kynuramine substrate). Rats received 100 mg/kg p.o. of each compound and MAO activity was determined 2, 4 and 7 days after treatment. Each value is the mean of at least two observations.

tissues. Doses beyond 1×10^{-4} were not tried as precipitation of the compounds occurred on incubation (Table 4). 3AO, even at higher doses of 1×10^{-3} was ineffective in producing any inhibition. The absence of *in vitro* MAO inhibitory activity and powerful *in vivo* activity of these compounds would suggest a metabolic transformation of these compounds *in vivo*. Dubrick *et al.*[12] have shown that 2, methyl-3-piperidinopyrazine, a weak MAO inhibitor *in vitro*, is converted into a much more potent inhibitor *in vivo*. In mice the conversion is known to be blocked by SKF525A. In our experiments on rats, pretreatment of rats with SKF525A failed to show any effect on the MAO inhibition produced by these compounds, except compound 3AO, A and E where some protection on the brain inhibition was seen after pretreatment with SKF525A. Significant effect was, however, seen only with compound A (Table 5).

Effect on levels of noradrenaline, dopamine and 5-hydroxytryptamine in the rat. A single oral dose (100 mg/kg) of all the four compounds produced a significant increase in the levels of cerebral noradrenaline. Dopamine levels were, however, increased significantly by compounds A and C. The effect on the peripheral tissues like the heart, was less marked. Significant increase was only seen with compound E (Fig. 9). The effect of two different doses of 3AO on the noradrenaline, dopamine and 5HT content is shown in Fig. 10 and Table 6. As can be seen from the table, both the doses of 3AO increased significantly the levels of noradrenaline and dopamine in the brain. Only

TABLE 4. EFFECT OF 3AO, TRANYLCYPROMINE, COMPOUNDS A, B (PARGYLINE), C, D AND E ON *in vitro* INHIBITION OF BRAIN AND LIVER MONOAMINE OXIDASE

Test substance	Dose	Inhibition (%)			
		5HT (substrate)		Kynuramine (substrate)	
		Brain	Liver	Brain	Liver
3AO	1×10^{-6}	—	—	0	0
	$1{\cdot}5 \times 10^{-6}$	—	—	0	4
	1×10^{-3}	0	0	—	—
Tranylcypromine	1×10^{-6}	94	98	52	79
*A	1×10^{-4}	39	45	21	26
	4×10^{-7}	56	—	—	—
B	1×10^{-6}	89	96	63	80
(pargyline)	$1{\cdot}5 \times 10^{-6}$	—	—	79	—
*C	1×10^{-4}	4	0	21	18
*D	1×10^{-4}	15	20	23	22
*E	1×10^{-4}	11	7	19	19

Each value is the mean of at least two observations.
* Doses beyond 1×10^{-4} were not tried because of the precipitation of the compound in the homogenates on incubation.

TABLE 5. EFFECT OF SKF525A ON THE INHIBITION OF RAT BRAIN AND LIVER MAO BY 3AO AND COMPOUNDS A, C, D AND E

Compound	Dose mg/kg p.o.	Pretreatment with SKF525A (50 mg/kg i.p.) 1 hr before the compound	Inhibition (%)	
			Brain	Liver
3AO	2	—	52 ± 9 (3)	83 ± 3 (3)
3AO	2	50	38 ± 12 (3)	84 ± 3 (3)
A	50	—	86 ± 2 (3)	95 ± 0·3 (3)
A	50	50	45 ± 13* (3)	88 ± 3 (3)
C	50	—	89 ± 4 (3)	95 ± 2 (3)
C	50	50	89 ± 5 (3)	95 ± 0·7 (3)
D	50	—	83 ± 4 (2)	93 ± 1 (2)
D	50	50	73 ± 4 (3)	94 ± 0·7 (3)
E	100	—	94 ± 0·7 (3)	96 ± 0·3 (3)
E	100	50	74 ± 16 (3)	93 ± 3 (3)

All animals were killed 6 hr after giving the drug.
* $P < 0{\cdot}05$.

with higher doses a significant effect on the heart noradrenaline was seen. Levels of 5-hydroxytryptamine in the brain were also increased significantly by 3AO and pargyline (Table 6). None of the compounds had any effect on the uptake of infused noradrenaline in the rat heart, except compound E (Table 7).

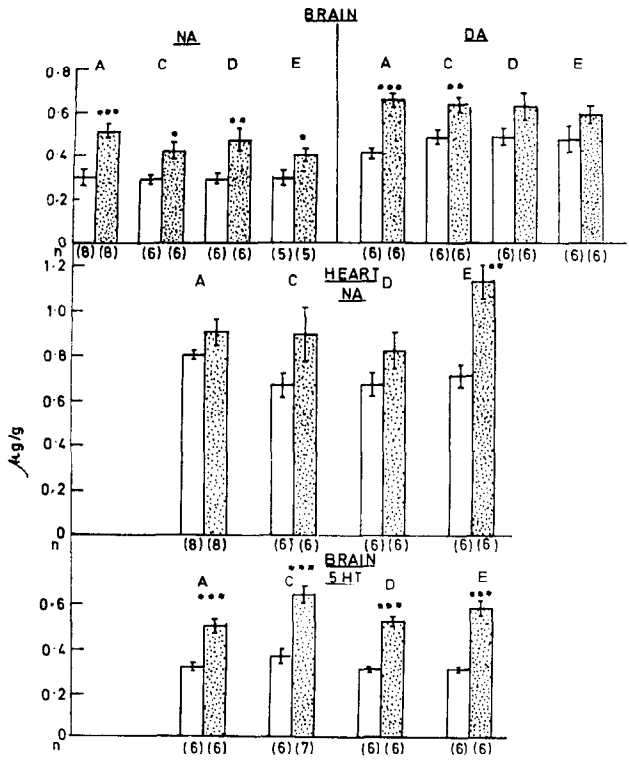

FIG. 9. The effect of compounds A, C, D and E (100 mg/kg, 16 hr after) on the levels of noradrenaline (NA), dopamine (DA) and 5-hydroxytryptamine (5HT) levels in the rat brain. Open columns (controls) and crossed columns (treated). Figures in the parenthesis indicate the number of observations.

* P < 0·05.
** P < 0·01.
*** P < 0·001.

TABLE 6. EFFECT OF 3-AMINO-2-OXAZOLIDINONE (3AO) AND PARGYLINE ON THE NORADRENALINE, DOPAMINE AND 5-HYDROXYTRYPTAMINE CONTENT OF THE RAT TISSUES

Test substance and dose (mg/kg)	Increase from the control (%)			
	Noradrenaline		Dopamine	5-Hydroxytryptamine
	Brain	Heart	Brain	Brain
3-amino-2-oxazolidinone				
5	46 (4)‡	1 (5)	40 (10)*	78 (5)†
100	65 (8)‡	33 (8)†	50 (5)†	—
Pargyline				
100	59 (7)†	16 (10)*	22 (7)	138 (6)‡

The figures in the parentheses indicate the number of observations. Animals killed 16 hr after treatment.

* P < 0·05.
† P < 0·01.
‡ P < 0·001.

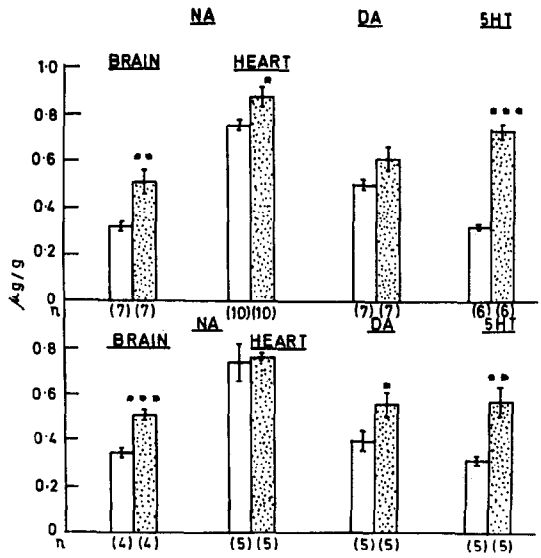

FIG. 10. The effect of pargyline (100 mg/kg p.o. after 16 hr—upper column) and 3AO (5 mg/kg p.o. after 16 hr—lower column) on the levels of noradrenaline (NA), dopamine (DA) and 5-hydroxytryptamine (5HT) levels in the rat brain. Open columns (controls) and crossed columns (drug treated).

* $P < 0{\cdot}05$.
** $P < 0{\cdot}01$.
*** $P < 0{\cdot}001$.

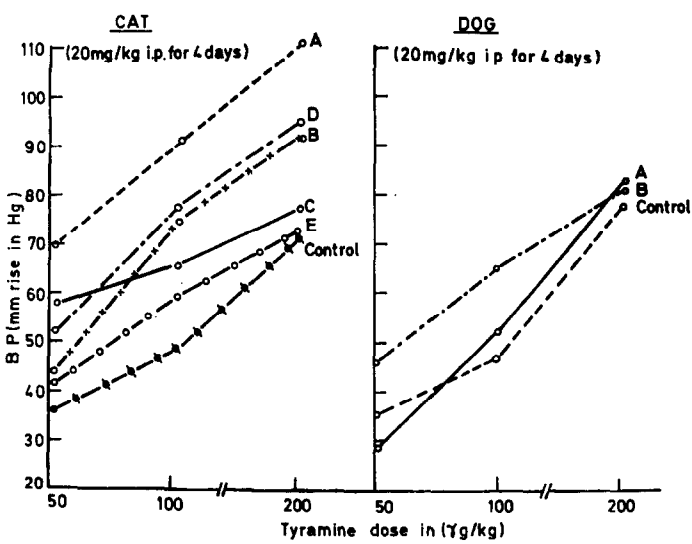

FIG. 11. The actions of compounds A, C, D and E on the blood pressure response to tyramine. Ordinate, increase in blood pressure. Abscissa, dose of tyramine (μg/kg). The animals were pretreated with 20 mg/kg p.o. for 4 days. Note the marked increase in the pressor responses to tyramine in the case of the cat. In the case of the dog, however, the potentiation effect was less marked.

TABLE 7. EFFECT OF COMPOUNDS A, C, D, E (30 mg/kg p.o.) AND COCAINE (20 mg/k p.o.) ON THE NORADRENALINE UPTAKE IN THE RAT HEART

	Treatment	Noradrenaline infused (μg)	Noradrenaline uptake (%)
1	Control	20	100
2	A	20	81
3	C	20	100
4	D	20	100
5	E	20	30
6	Cocaine	20	20

Noradrenaline was infused 2 hr after treatment with the drug and the animals were killed 5 min after the infusion was given.

Potentiation of the action of tyramine. The actions of compounds A, B, C, D and E on the blood pressure responses to tyramine are shown in Fig. 11. The dose–response curves for tyramine were markedly shifted to the left after treatment with these four compounds. The potency of these in descending order was A, D, B. The effect on the dog was, however, less marked as compared to the effect on the cat (Fig. 11). A marked potentiation of tyramine response was also seen in rats and the potentiating effect was seen both on the duration and intensity of the tyramine response (Fig. 12).

Bretylium like action of compound A and B. Pretreatment of rats with compound A completely prevented the depletion of heart noradrenaline induced by guanethidine (Table 8). Pargyline is also known to antagonise the depletion produced by guanethidine.[13] The effect of compound A and B on the rise in blood pressure obtained upon electrical stimulation of the distal end of the splanchnic nerve was studied. Both compound A and B blocked the hypertension induced by low frequency stimulation (5 cycles/sec), but the hypertension induced by high frequency stimulation was blocked by 33 and 55 per cent respectively (mean of four experiments). Compound A blocked the hypertension induced by DMPP by about 81 per cent (mean of four experiments). In the case of compound B however, the effect was biphasic, a reversal of the pressor response followed by a slight rise.

TABLE 8. ANTAGONISM OF THE GUANETHIDINE INDUCED DEPLETION OF HEART NORADRENALINE BY COMPOUND A

Treatment	Heart noradrenaline (μg/g)
Controls	0·75 ± 0·03
Guanethidine	0·44 ± 0·03
Compound A + Guanethidine	0·74 ± 0·04

Rats were first treated with compound A (50 mg/kg p.o.) and 2 hr later guanethidine (10 mg/kg i.p.) was given and the animals killed 5 hr later.

DISCUSSION

The pharmacological and biochemical data presented in this paper show that 3AO and some of its condensation products are a potent group of antihypertensive agents

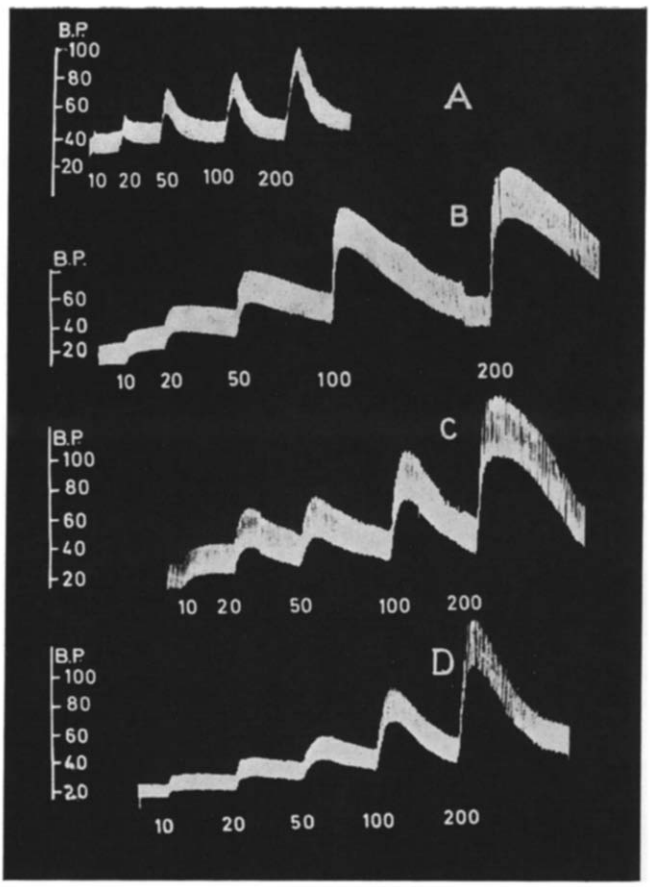

FIG. 12. Blood pressure responses to tyramine in pithed rats. A, Control response to different doses of tyramine; B, Compound A 100 mg/kg p.o.; C, Compound B 100 mg/kg p.o.; D, Compound A 30 mg/kg p.o. All compounds were given for 4 days and experiment was done on the 5th day. Note the marked increase in the pressor response of tyramine after treatment with compound A and B. Both the duration and intensity of the response was increased.

with powerful MAO inhibiting properties *in vivo* but not *in vitro*. Although the antihypertensive activity of some of these compounds was three times that of pargyline, there was no clear-cut correlation between the MAO inhibition and the antihypertensive effect, e.g. maximum inhibition of the MAO was seen 6–12 hr after treatment with the compound but the maximum fall of blood pressure was seen only after a few days of treatment. Again in the case of 3AO, low doses of the compound which produce maximum inhibition of MAO did not produce any significant fall of blood pressure (Fig. 3). Like many other MAO inhibitors some of these compounds showed bretylium like action. Compound A prevented the depletion of heart catecholamine by guanethidine (Table 8) and prevented electrical or DMPP induced sympathetic stimulation from exerting an effect on the blood vessels. Since the sympatholytic action of these compounds is not due to the blocking action of catecholamine on receptor sites or to interference with transmission in sympathetic ganglia, it is possible that these compounds prevent the nerve impulses from releasing noradrenaline on the receptor sites. This is one of the mechanisms postulated by which MAO inhibitors lower the blood pressure.[13]

The effect of these compounds on *in vivo* MAO inhibition indicates that many of the compounds are two to three times more active than pargyline in inhibiting brain and liver MAO. The inability of these compounds to produce any MAO inhibition *in vitro* suggests that MAO inhibition *in vivo* may be primarily due to a metabolite formed *in vivo*. However, SKF525A exerted no measurable effect on the MAO inhibition (*in vivo*) produced by subsequent administration of these compounds. In the case of compounds A, E and 3AO, however, there was some protection in brain by pretreatment with SKF525A. Significant difference was only seen in the case of compound A. These studies would probably indicate that the MAO inhibition is produced by a metabolite, the formation of which is not blocked by SKF525A. Similar observations have been reported by Squires and Lassen (1968)[14] as regards NSD 2023 which was a much more effective inhibitor of mouse brain MAO *in vivo* than *in vitro*.

These compounds show typical effects of MAO inhibitors in experimental animals. They all increased the levels of brain biogenic amines (Figs. 9 and 10), antagonized reserpine induced hypothermia, gave a positive dopa test (unpublished observations). They potentiated the effects of adrenaline, noradrenaline and tyramine on the blood pressure of experimental animals (Figs. 11, 12). Despite the fact that these compounds showed good antihypertensive activity, they were not tried in the clinic because of the problems encountered with MAO inhibitors (hypertensive crisis, etc.).

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