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Inhibition of dopadecarboxylase in the rat by a series of benzyloxyamines

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A series of eleven benzyloxyamines has been evaluated for *in vitro* and *in vivo* inhibition of dopa decarboxylase. The effect of these inhibitors on the rise in [^{14}C]amines in the brain after [^{14}C]dopa has also been assessed. Of these compounds, 3,4-dihydroxybenzyloxyamine appears to be a potent selective peripheral inhibitor of dopa decarboxylase. Its activity is similar to that of Ro 4-4602 [N^1-(DL-seryl)-N^2-(2,3,4-trihydroxybenzyl) hydrazine].

Attempts to reduce the concentration of endogenous brain catecholamines by inhibiting the enzyme dopa decarboxylase (DDC) have been unsuccessful except in the presence of excess substrate. When this condition exists the enzyme becomes rate controlling and DDC inhibitors at high concentrations will limit the rise in brain catecholamines (Bartholini & Pletscher, 1969). At lower doses the DDC inhibitors may actually potentiate the rise in brain catecholamines after L-dopa (Bartholini & Pletscher, 1968). Decarboxylation in peripheral organs, particularly intestines, liver and kidney, is the major route of metabolism of this amino-acid in most mammals (Davis & Awapara, 1960). DDC inhibitors can thus significantly potentiate the blood and tissue concentrations of dopa and increase its half-life. Those inhibitors which do not readily pass the blood-brain barrier will not, of course, markedly inhibit the central enzyme and decarboxylation will proceed relatively unimpaired resulting in a higher brain catecholamine concentration.

Ro 4-4602 [N^1-(DL-seryl)-N^2-(2,3,4-trihydroxybenzyl) hydrazine] and MK 485 [DL-α-methyl-α-hydrazino-3,4-dihydroxybenzylpropionic acid], potent decarboxylase inhibitors with a selective peripheral action, produce enhanced catecholamine concentrations in the brains of rats given L-dopa (Bartholini & Pletscher, 1968; 1969). Administration of these compounds to patients undergoing treatment with L-dopa for parkinsonism has allowed a substantial reduction in the dose of the amino-acid with consequent reduction in side-effects (Cotzias, Papavasiliou & Fellene, 1969; Tissot, Galliard & others, 1969).

A series of benzyloxyamine derivatives has been examined for selective inhibition of peripheral DDC in the rat. We report here their effect on the concentration of [^{14}C] labelled acids and amines in the heart and brain of rats given L-[^{14}C]dopa.

MATERIALS AND METHODS

Materials

L-[3-^{14}C] (3,4-Dihydroxyphenyl)alanine was obtained from the Radiochemical Centre, Amersham, Bucks. All the benzyloxyamines were synthesized in these laboratories (Drain, Williams & Howes, 1964; Drain, Howes & Williams, 1965a; 1965b). Ro 4-4602 was a gift of Professor A. Pletscher, Hoffmann la Roche Ltd., Basle, Switzerland. All other chemicals used were commercial analytical grade reagents.

The rats were Wistar (SNR strain, male, 70–130 g). They were fed Dixons FFG diet which was not withheld before any experiments.

Methods

DDC inhibition *in vitro* was measured manometrically (Hartman, Akawie & Clark, 1958) using a 3000 *g* supernatant of a fresh guinea-pig kidney homogenate as the source of enzyme.

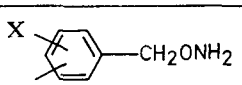
Inhibition of dopamine β-oxidase (DBO) *in vitro* was measured by the method of Creveling, Daly & others (1962). The phosphate buffer was passed through a Zeo-Carb 225 K^+ column to remove heavy metal ions which interfere with the reaction.

In vivo decarboxylase inhibition was measured in kidney extracts from rats killed by decapitation at various times after the enzyme inhibitor had been administered. Control animals received saline. The kidneys were removed, homogenized and incubated with 5-hydroxytryptophan (5-HTP) as substrate. The 5-hydroxytryptamine (5-HT) formed was measured according to the method of Bogdanski, Weissbach & Udenfriend (1957).

[^{14}C]Catecholamine concentrations in the hearts and brains of rats receiving [^{14}C]dopa and DDC inhibitors were measured by a method adapted from Bartholini & Pletscher (1968). Rats were dosed with the test compound and after 30 min L-[^{14}C]dopa (specific activity 1·25 μCi/mg) was administered intraperitoneally. One h later the rats were decapitated, the brains and hearts removed and the organs individually homogenized in 5 ml 0·4N perchloric acid. The homogenate was adjusted to pH 5–6 with 5N potassium hydroxide using Universal Indicator internally, cooled, and the potassium perchlorate formed removed by centrifugation.

The supernatant (about 6 ml) was applied to a 1 cm × 2 mm Dowex 50 × 4, (200–400 mesh) column in the H^+ form, washed on with two × 2 ml distilled water followed by 5 ml 0·05M phosphate buffer pH 6·5. The total eluate (about 15 ml) contained both [^{14}C]phenolcarboxylic- and amino-acids derived from [^{14}C]dopa administered to the animals. The [^{14}C]catecholamines were eluted from the column with 6 ml M potassium chloride in 0·01N hydrochloric acid. 2 ml fractions of the acid and amine fractions were added to 20 ml scintillator solution and the activity measured in a liquid-scintillation counter.

Table 1. *Structure of benzyloxyamines and their* in vitro *enzyme inhibition.*



Compound No.	X	Y	Dopa decarboxylase *in vitro* RM* × 10^{-3} to give 50% inhibition	Dopamine β-oxidase *in vitro* inhibition at 1/10 RM*
SNR 1635	H	H	10	73
SNR 1514	2OH	H	1	8
NSD 1024	3OH	H	0·67	63
SNR 1515	4OH	H	3·3	93
SNR 1516	2OH	5Cl	3·9	9
NSD 1050	3OH	4Cl	0·11	50
NSD 1055†	3OH	4Br	0·13	41
NSD 1029	3OH	6Cl	2	10
SNR 1639	4OH	3Cl	0·63	95
SNR 1517	4OH	3Br	0·98	89
SNR 1531	3OH	40H	0·33	82

* RM = Relative Molarity.

† Official name Brocresine.

In some experiments the rats were dosed orally with the inhibitor twice daily for three days followed by a single dose on the morning of the fourth day. The procedure for the single dose experiments was then followed.

RESULTS AND DISCUSSION

The activities of the benzyloxamines as inhibitors of DDC and DBO are shown in Table 1. We have found (unpublished) that these compounds do not markedly interfere with other enzymes associated with catecholamine metabolism. In particular, even at high concentration they show no appreciable inhibition of monoamine oxidase *in vitro*. Against tyrosine hydroxylase most of the benzyloxyamines are inactive *in vitro*, a few, notably SNR 1531, displaying weak inhibition of this enzyme. Three of the compounds, NSD 1024, NSD 1055 and SNR 1531 have been tested against catechol *O*-methyl transferase and only SNR 1531 showed any inhibition (27% at 1/5 Relative Molarity). The inhibition by SNR 1531 of tyrosine hydroxylase and catechol *O*-methyl transferase is probably due to non-specific effects of the catechol group rather than the oxyamine part of the molecule (Bacq, Gosselin & others, 1959; McGeer & McGeer, 1967).

The effect of the DDC inhibitors given intraperitoneally on the rise in [^{14}C]labelled acids and amines in the brains of rats receiving [^{14}C]dopa is shown in Table 2. Since

Table 2. [^{14}C]*Acid and amine concentrations in brains of rats given* [^{14}C]*dopa and various doses of decarboxylase inhibitors intraperitoneally.*

Compound No.	[^{14}C] acids in n mol/g brain Dose of inhibitor (mg/kg)			[^{14}C] amines in n mol/g brain Dose of inhibitor (mg/kg)		
	10	33	100	10	33	100
SNR 1635	9·2	10·5	18·1	0·67	0·84	1·62
SNR 1514	15·7	22·1	31·5	2·11	3·21	4·17
NSD 1024	16·2	21·8	34·1	2·66	2·57	2·87
SNR 1515	10·5	9·9	12·1	0·62	0·61	0·85
SNR 1516	9·9	13·4	27·0	0·94	0·99	2·06
NSD 1050	11·3	13·2	14·9	1·46	1·89	1·98
NSD 1055	10·0	12·0	16·1	0·88	1·28	1·45
NSD 1029	11·9	24·0	30·5	1·31	2·04	1·97
SNR 1639	11·8	14·0	27·6	0·57	0·62	1·34
SNR 1517	9·5	11·8	19·3	0·60	0·78	0·94
SNR 1531	30·7	35·8	38·7	5·24	5·21	3·67
Ro 4-4602	29·5	32·0	34·0	4·04	4·72	3·79
Controls		9·2 (±0·34)*			0·64 (±0·02)*	
Ro 4-4602 at 50 mg/kg		37·3 (±1·1)*			4·82 (±0·12)*	

Rats received decarboxylase inhibitor i.p. and 30 min later L[^{14}C]dopa (16 mg/kg) i.p. After a further 60 min the animals were killed and the [^{14}C]acid and [^{14}C]amine concentration in the brains determined. The figures are the means of two determinations at each dose (*s.e. of 26 determinations).

they block the major route of metabolism of dopa, thereby potentiating its blood concentrations, efficient *in vivo* DDC inhibitors will cause a large increase in the concentration of labelled acids in the brains of rats receiving [^{14}C]dopa. The concentration of labelled amine achieved in the brain will depend on the concentration of labelled amino-acid and on the amount of DDC inhibition. Thus it would be expected that the brain [^{14}C]labelled amine concentration, would initially increase with inhibitor dose and then decrease as the central DDC becomes progressively inhibited. This condition has been demonstrated with SNR 1531 and Ro 4-4602. Central inhibition

becomes noticeable only at high doses of the inhibitors. With the other compounds a plateau rather than a point of inflection was reached. The lower concentrations of labelled acids and amines in the brains of rats receiving the other compounds intraperitoneally before [^{14}C]dopa indicate that these other benzyloxyamines are less efficient DDC inhibitors than SNR 1531 *in vivo*. Several compounds that are potent *in vitro* DDC inhibitors do not appear to be very active *in vivo* (NSD 1050, NSD 1055 and SNR 1517) whereas some compounds of only modest *in vitro* activity appear to be fairly potent *in vivo* (NSD 1029 and SNR 1514). Compounds which produced the biggest increases in [^{14}C]labelled acid concentrations are those compounds which have been shown by Hansson, Fleming & Clark (1964) to be efficient inhibitors of formation of $^{14}CO_2$ from [^{14}C]carboxyl-labelled dopa in the mouse.

The more active compounds were tested to find their effect on kidney DDC after single oral or intraperitoneal administration to the rat (Table 3). Only SNR 1531 and Ro 4-4602 showed comparable inhibition by either route, other compounds being less active orally. After repeated oral dosing with DDC inhibitors the effect of a single intraperitoneal injection of [^{14}C]dopa on [^{14}C]labelled acid and amine concentrations in the brains of rats was assessed (Table 4). In rats that received several doses of either NSD 1024 or SNR 1514 before [^{14}C]dopa, the concentrations of labelled acids and amines in the brains were not greatly potentiated except at very high concentrations of inhibitor. NSD 1055 showed better potentiation after multiple oral rather than single intraperitoneal administrations. NSD 1055, which is also a powerful inhibitor of

Table 3. *Kidney decarboxylase inhibition measured after oral and intraperitoneal doses of the enzyme inhibitor.*

Compound No.	Inhibition (%) 90 min after 10 mg/kg i.p.	Inhibition (%) at various times after 10 mg/kg orally 1 h	2 h	4 h	5½ h
SNR 1514	72 (69–76)	7 (0–12)	3 (0–9)	11 (3–17)	10 (3–19)
NSD 1024	64 (54–75)	13 (8–15)	25 (8–48)	16 (15–18)	22 (16–30)
NSD 1055	49 (43–55)	25 (10–49)	18 (13–23)	15 (0–34)	4 (0–7)
SNR 1531	77 (76–82)	46 (36–59)	60 (50–66)	66 (60–71)	63 (48–78)
Ro 4-4602	77 (73–82)	76 (72–80)	78 (75–80)	77 (76–78)	76 (75–77)

Groups of 4 rats were killed at various times after administration of the enzyme inhibitor. The kidneys were removed, homogenized and incubated with 5-hydroxytryptophan as substrate. The 5-hydroxytryptamine produced was estimated. The figures in the brackets indicate the range.

Table 4. [^{14}C]*Acid and* [^{14}C]*amine concentrations in brains of rats given* [^{14}C]*dopa i.p. and repeated oral doses of various decarboxylase inhibitors.*

Compound No.	[^{14}C]Acids in nmol/g brain Dose of inhibitor (mg/kg) 3·3	10	33	100	[^{14}C]Amines in nmol/g brain Dose of inhibitor (mg/kg) 3·3	10	33	100
SNR 1514	11·5	10·5	13·4	24·1	0·60	0·54	0·74	2·30
NSD 1024	8·9	8·9	10·8	17·6	0·53	0·34	0·94	3·96
NSD 1055		10·3	25·5	30·8		1·21	1·77	3·62
SNR 1531	15·0	26·0	34·4	40·4	1·76	3·73	5·42	5·38
Controls		9·0 (±0·6)*				0·65 (±0·05)*		
Ro 4-4602 at 50 mg/kg i.p.		38·3 (±2·1)*				5·68 (±0·25)*		

Groups of three rats were given 8 oral doses of inhibitor in 5 days. 30 min after the last dose the rats received [^{14}C]dopa (16 mg/kg) i.p. and were killed 1 h later. The [^{14}C] acid and [^{14}C] amine concentrations in the brain were measured. (*s.e. of 12 determinations.)

histidine decarboxylase, has been shown by Levine (1966) to produce its maximum clinical effect against this enzyme after 2–3 weeks oral dosing, an observation supported by the results here. SNR 1531 multiple dosed rats showed labelled amine concentrations comparable with those seen in the single dosing schedule, indicating that there does not appear to be a build-up in the central DDC inhibition with this compound on multiple dosing.

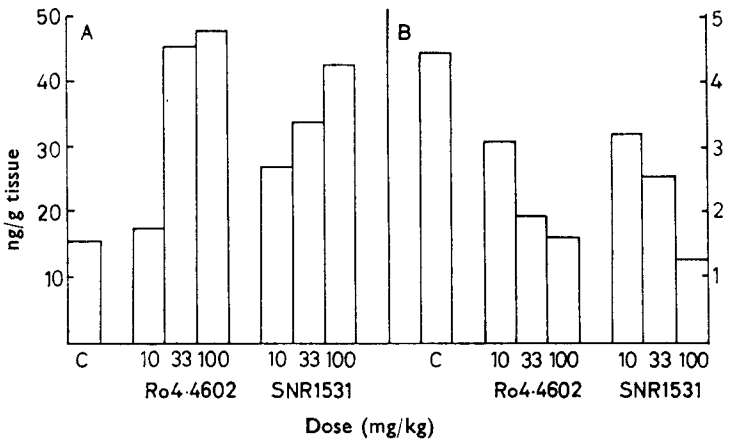


FIG. 1. [^{14}C]Acid and [^{14}C]amine concentrations in hearts of rats given [^{14}C]dopa and either Ro 4-4602 or SNR 1531. Groups of four rats received decarboxylase inhibitor i.p. and 30 min later L-[^{14}C]dopa (16 mg/kg) i.p. After a further 60 min the animals were killed and the [^{14}C]acid (A) and [^{14}C]amine (B) concentrations in the hearts measured. Controls (C) received saline and L-[^{14}C]dopa.

Fig. 1 shows the effect of single intraperitoneal doses of SNR 1531 or Ro 4-4602 on the labelled acid and amine concentrations in the hearts of rats also receiving [^{14}C]dopa. In the heart a dose-related decrease in the labelled amine concentration is seen at the same time as the labelled acid concentration is increasing. Clearly, in the heart, there is good penetration of inhibitor causing a marked inhibition of DDC. An experiment

Table 5. [^{14}C]*Acid and* [^{14}C]*amine concentrations (nmol/g) in brains of rats given* [^{14}C]*dopa and either SNR 1531 or Ro 4-4602.*

Dose of compound in mg/kg weight	SNR 1531		Ro 4-4602	
	[^{14}C]Acids	[^{14}C]Amines	[^{14}C]Acids	[^{14}C]Amines
None	8·8	0·50		
20	29·8	4·9	38·4	3·9
30	32·8	4·2	35·1	4·1
45	33·0	4·2	38·2	4·5
65	32·2	3·8	41·5	3·6
100	25·1	3·3	28·3	2·3

Groups of 2 rats received decarboxylase inhibitor i.p. and 30 min later L-[^{14}C]dopa (16 mg/kg) i.p. After a further 60 min the animals were killed and the [^{14}C]acid and [^{14}C]amine concentrations in the brains measured.

designed to detail more closely the relation between the dose of SNR 1531 or Ro 4-4602 and the labelled acid and amine concentration in the brains of rats receiving inhibitor and [^{14}C]dopa is reported in Table 5. Both compounds show greatly increased labelled amine concentrations and the absence of marked inhibition of DDC except at very high doses of inhibitor.

It is evident that SNR 1531 is the most effective benzyloxyamine DDC inhibitor studied. The good inhibition seen in peripheral tissues at concentrations which do not appear to affect the brain enzyme indicate that SNR 1531 may find therapeutic application as an adjunct to L-dopa in the treatment of Parkinson's disease.

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