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A note on the blockade of uptake of noradrenaline by 4-chloro-αα-dimethylphenethylaminopropan-2-one in rodents

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4-Chloro-αα-dimethylphenethylaminopropan-2-one blocked uptake of [^{3}H]noradrenaline in the heart, had no effect on the endogenous levels of catecholamines or 5-hydroxytryptamine in the heart, brain or adrenals and decreased the [^{3}H]noradrenaline-depleting activity of metaraminol, but not reserpine, in rodents. The compound appears to act by interfering with the active transport of noradrenaline through the nerve cell membrane.

VARIOUS 4-chlorinated aralkylamines cause alterations in the tissue levels of the monoamines. 4-Chloro-*N*α-dimethylphenethylamine decreases the brain 5-hydroxytryptamine (5-HT) concentration without appreciable effect on the noradrenaline concentration in the rat (Pletscher, Bartholini & others, 1964; Lippmann & Wishnick, 1965a) or guinea-pig, whereas in the mouse and rabbit, there is but little effect on either concentration (Pletscher & others, 1964). In the brain of the cat, 4-chloro-*N*α-dimethylphenethylamine causes a large decline in the 5-HT concentration, but not in the noradrenaline concentration, in the hypothalamic area and in the diencephalo-thalamic area (Lippmann & Wishnick, 1965a). 4-Chloro-αα-dimethylphenethylamine causes no decrease in 5-HT or noradrenaline in the brain of the mouse or guinea-pig (Dubnick, Leeson & others, 1963). In the rat brain after administration of di(3,4-dichlorophenethyl)amine, there is a rapid transient decrease of 5-HT, noradrenaline and dopamine as well as a concomitant increase of 5-hydroxyindolylacetic acid (5-HIAA). After treatment with *N*α-dimethyl-4-nitrophenethylamine, a rapid transient decrease of 5-HT and 5-HIAA occurs in the rat brain (Pletscher, Da Prada & others, 1966). In the rat and guinea-pig brain the decrease in 5-HT after 4-chloro-*N*α-dimethylphenethylamine is slow and is accompanied by a slow decrease in 5-HIAA (Pletscher & others, 1964). Studies have been made on the structurally related 4-chloro-aralkylamine compound 4-chloro-αα-dimethylphenethyl aminopropan-2-one (AY-14,948) and this report describes the effects of AY-14,948 on the uptake and storage of the monoamines.

EXPERIMENTAL

For the determination of radioactive noradrenaline levels in tissues, male albino mice (Canadian Breeding Laboratories, 24–26 g) or male albino rats (Charles River, 60–80 g) were injected in the tail vein with 0·25 ml containing 5 μc (±)[7-^{3}H] noradrenaline* hydrochloride (New England Nuclear Corp.) and 0·85 μg (−)-noradrenaline hydrochloride in a solution of 0·75% sodium chloride and 0·01N hydrochloric acid. Drugs were injected intraperitoneally in 0·5 ml saline unless otherwise specified. Control animals received injections of the appropriate vehicle. The

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* 2-Amino-1-(3,4-dihydroxyphenyl)-[1-^{3}H]ethanol.

tissue samples were homogenized in ice-cold 0·4N perchloric acid and centrifuged. A portion of the supernatant fluid was transferred to a vial containing a mixture of 1 ml methanol, 3 ml ethanol and 10 ml toluene phosphor [0·4% of 2,5-diphenyloxazole and 0·005% of 1,4-di(5-phenyloxazol-2-yl)benzene], and the total radioactivity was measured by liquid scintillation counting. The counting efficiency was 22%. Brain catecholamine levels were determined in the rats as described by Lippmann & Wishnick (1965b). Brain 5-HT levels were estimated by the fluorimetric procedure of Bogdanski, Pletscher & others (1956) on aliquots of the final acid extract (Mead & Finger, 1961). The levels of heart noradrenaline in acetic acid eluates from aluminium oxide columns were determined by oxidation with ferricyanide (Whitby, Axelrod & Weil-Malherbe, 1961). Adrenal catecholamines were isolated and determined as previously described (Lippmann & Wishnick, 1965b). Monoamine oxidase activity was measured by the method of Kraml (1965) and catechol-*O*-methyl transferase according to Anderson & D'Iorio (1966).

Drugs used were: reserpine (Serpasil; Ciba Co. Ltd.) and metaraminol bitartrate (Aramine; Merck Sharpe and Dohme Ltd.). AY-14,948 was synthesized by Dr. A. Langis (Ayerst Laboratories) and was in the form of the hydrochloride salt. Student's *t*-test was used to evaluate the data.

TABLE 1. EFFECT OF AY-14,948 ON THE UPTAKE AND RELEASE OF [^{3}H]NORADRENALINE IN THE MOUSE AND RAT HEART

A. MOUSE

Drug	Time drug given before or after [^{3}H]noradrenaline	Radioactivity content	
		Counts/min/g ± s.e.	% of control
None	1 hr, before	4,394 ± 197	
AY-14,948 ..	1 hr, before	2,516 ± 94	58
None	1 hr, after	3,551 ± 163	
AY-14,948 ..	1 hr, after	3,285 ± 126	93

AY-14,948 was administered at 50 mg/kg, i.p. The animals were killed 4 hr after the drug. There were 27 animals in the control and 21 in the treated group.

B. RAT

None	1 hr, before	16,193 ± 1,461	
AY-14,948 ..	1 hr, before	5,029 ± 453	31
Imipramine ..	1 hr, before	796 ± 69	5
None	1 hr, after	15,588 ± 956	
AY-14,948 ..	1 hr, after	15,316 ± 785	96
Imipramine ..	1 hr, after	16,596 ± 843	106

AY-14,948 was administered at 50 mg/kg, i.p., and imipramine at 20 mg/kg, i.p. The animals were killed 4 hr after the drug. There were 12 animals in the control and 10 in the treated group.

RESULTS

The effects of AY-14,948 on the uptake and release of [^{3}H]noradrenaline ([^{3}H]-NA) in the mouse and rat heart are shown in Table 1. AY-14,948 was administered (50 mg/kg, i.p.) to the animals one hr before or after [^{3}H]-NA and the radioactivity contents of the hearts determined. When the AY-14,948 was given before the [^{3}H]-NA and the animals were killed 4 hr after administration of the aralkylamine, the radioactivity of the mouse and rat heart had decreased by 42 and 69% respectively.

No appreciable decrease in [^{3}H]-NA level was observed in either the mouse or rat heart when AY-14,948 was given after it. In the rat, imipramine (20 mg/kg, i.p.) caused a 95% drop in the [^{3}H]-NA when given before and no decline when given after it. Thus, AY-14,948, like imipramine, caused a decline in the uptake and had no effect on the release of [^{3}H]-NA in the heart.

TABLE 2. EFFECT OF AY-14,948 ON CATECHOLAM NE AND 5-HT CONTENTS OF VARIOUS TISSUES OF THE RAT

Drug	Time animals killed after AY-14,948, hr	Heart catecholamines, μg/g ± s.e.	P	Adrenal catecholamines, μg/pair ± s.e.	P
None ..		0·52 ± 0·04 (13)		32·5 ± 1·5 (17)	
AY-14,948	7·5	0·61 ± 0·03 (7)	>0·1	29·1 ± 1·6 (8)	>0·1
AY-14,948	16·5	0·45 ± 0·06 (8)	>0·1	32·3 ± 2·3 (7)	>0·5
AY-14,948	24·0	0·52 ± 0·03 (8)	>0·5	25·6 ± 3·1 (6)	>0·05
		Brain catecholamines, μg/g ± s.e.		Brain 5-HT μg/g ± s.e.	
None ..		0·58 ± 0·02 (7)		0·61 ± 0·05 (7)	
AY-14,948	7·5	0·59 ± 0·03 (8)	>0·5	0·56 ± 0·06 (8)	>0·1
AY-14,948	16·5	0·53 ± 0·02 (7)	>0·1	0·64 ± 0·08 (8)	>0·5
		Heart catecholamines, μg/g ± s.e.		Adrenal catecholamine, μg/pair ± s.e.	
None ..		0·65 ± 0·07 (13)		21·9 ± 0·6 (15)	
AY-14,948	3	0·58 ± 0·04 (8)	>0·1	20·6 ± 0·7 (8)	>0·1
AY-14,948	24	0·56 ± 0·05 (7)	>0·1	22·0 ± 1·7 (7)	>0·5
		Brain catecholamines, μg/g ± s.e.		Brain 5-HT μg/g ± s.e.	
None ..		0·42 ± 0·02 (15)		0·56 ± 0·02 (15)	
AY-14,948	3	0·36 ± 0·03 (8)	>0·1	0·57 ± 0·01 (8)	>0·5
AY-14,948	24	0·42 ± 0·02 (7)	>0·5	0·53 ± 0·03 (7)	>0·1

The animals were injected with AY-14,948 at 50 mg/kg, i.p. The tissues were removed at the times indicated. The number of animals is in parentheses.

Table 2 shows the effects of AY-14,948 on catecholamine and 5-HT contents of various tissues of the rat. There were no changes in the catecholamine contents of the heart, brain, or adrenals at 3, 7·5, 16·5 or 24·0 hr after administration of AY-14,948 (50 mg/kg, i.p.). There were also no changes in the 5-HT content of the brain under these conditions.

At a concentration of 1×10^{-4}M, AY-14,948 caused no inhibition of monoamine oxidase or catechol-*O*-methyl-transferase activities *in vitro*. One hr after the treatment of rats with AY-14,948 (50 mg/kg, i.p.) there was no inhibition of the monoamine oxidase activity in the brain or liver and no inhibition of the catechol-*O*-methyltransferase activity in the liver.

The effects of AY-14,948 on the activity of noradrenaline-releasing agents in the mouse heart were determined and are shown in Fig. 1. Mice received [^{3}H]-NA and 15 min later were given AY-14,948 (100 mg/kg, i.p.). Five min after the latter treatment the animals were injected with the noradrenaline releasers metaraminol (0·3 mg/mg, i.v.) or reserpine (0·5 mg/kg, i.v.). The animals were killed 1 hr after the initial treatment and the levels of noradrenaline in the hearts were determined. While AY-14,948 caused no appreciable change in the [^{3}H]-NA content, metaraminol and reserpine caused declines of 45 and 39%, respectively, in the

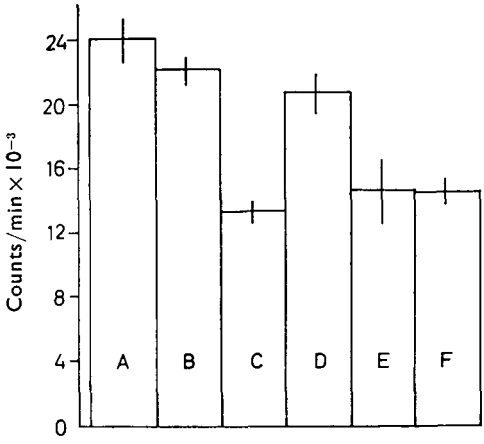

FIG. 1. Effects of AY-14,948 on the activity of noradrenaline-releasing agents in the mouse heart. A. Control. B. AY-14,948. C. Metaraminol. D. AY-14,948 + metaraminol. E. Reserpine. F. AY-14,948 + reserpine.

[^{3}H]-NA content in the heart. AY-14,948 significantly blocked the release obtained after metaraminol but not after reserpine.

DISCUSSION

AY-14,948 acts to block the uptake of [^{3}H]-NA into the storage sites and does not cause a release of the stored [^{3}H]-NA. In addition, it causes no changes in the endogenous levels of catecholamines in the heart, brain or adrenals and 5-HT in the brain. These activities of AY-14,948 are similar to those found with drugs such as imipramine and chlorpromazine. These latter drugs which cause a block in the uptake of [^{3}H]-NA (Axelrod, Hertting & Potter, 1962) also do not alter the endogenous catecholamine levels (Gey & Pletscher, 1961; Sulser & Brodie, 1961).

In comparison with other 4-chlorinated aralkylamines, AY-14,948 is similar to 4-chloro-$\alpha\alpha$-dimethylphenethylamine in its effect on noradrenaline in the rat heart, as both AY-14,948 (Table 2) and the latter compound (Dubnick & others, 1963) cause no changes in the endogenous level. In contrast, AY-14,948 does not decrease the brain 5-HT (Table 2), whereas 4-chloro-*N*α-dimethylphenethylamine does (Pletscher & others, 1964; Lippmann & Wishnick, 1965a). In the 4-chloro-α-methylphenethylamine series the methyl group on the carbon atom adjacent to the amino-function is necessary for the 5-HT-decreasing activity observed at 16 hr in rat brain. Compounds lacking this branching could be rapidly metabolized by monoamine oxidase (Pletscher & others, 1964, 1966; Fuller, Hines & Mills, 1965). Within this series the compound with the primary amino-group is the most effective in causing the decline in 5-HT. The most effective chloro-α-methylphenethylamine isomer is one in which there is a single chlorine atom substituted in the 4-position. The unchlorinated α-methylphenethylamines do not lower 5-HT levels. Noradrenaline

levels are not appreciably affected after administration of the chloro-α-methylphenethylamines under these conditions (Fuller & others, 1965). Other aralkylamines related to AY-14,948 have different activities in depleting brain noradrenaline and 5-HT, and are effective for varying durations. Di(3,4-dichlorophenethyl)amine, 4-chloro-*N*-(3,4-dimethylcyclohex-3-enylmethyl)phenethylamine and di-(-chlorophenethyl)amine decrease both the 5-HT and noradrenaline and only for a short time. *N*α-Dimethyl-4-nitrophenethylamine, 2-methyl-4-nitrophenethylamine and 3-chloro-*N*α-dimethylphenethylamine cause a drop in 5-HT of a short duration, and no fall in noradrenaline. 4-Chloro-*N*α-dimethylphenethylamine and di(4-chloro-α-methylphenethyl)amine decrease only the 5-HT, and for a long period, with no fall in noradrenaline (Pletscher & others, 1966). AY-14,948 thus also differs from these drugs in that it caused no decline in 5-HT or noradrenaline at any of the various time intervals.

AY-14,948 produces no inhibition *in vitro* or *in vivo* of the two major enzymes involved in the inactivation of the catecholamines, i.e. the monoamine oxidase and catechol-*O*-methyl transferase. In contrast, 4-chloro-*N*α-dimethylphenethylamine (Pletscher & others, 1966) causes inhibition of the monoamine oxidase *in vitro*.

The metaraminol-induced decrease in [^{3}H]-NA is blocked by a pretreatment with AY-14,948, but that caused by reserpine is not. Imipramine, desipramine and chlorpromazine block the uptake of noradrenaline and act by interfering with the active transport through the nerve cell membrane (Axelrod & others, 1962). Desipramine blocks the release of [^{3}H]-NA induced by metaraminol, but not that by reserpine (Stone, Porter & others, 1964; Carlsson & Waldeck, 1965). Thus, AY-14,948 is similar to desipramine in its actions and appears to act by interfering with the active transport through the nerve cell membrane.

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