(±)-(E)-2-(3,4-Dihydroxyphenyl)cyclopropylamine hydrochloride(ASL-7003): a rigid analogue of dopamine

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Many neurotransmitter substances, including dopamine, have been subjected to various physical analyses and theoretical calculations to determine their preferred conformation in various physical environments. Similarly, the synthesis of rigid analogues that are conformationally restricted has become an extensively used method of studying the interaction of neurotransmitters with their biological receptors.

Considerable theoretical and physical evidence (Bergin & Carlström, 1968; Bustard & Egan, 1971; Rekker, Engel & Nys, 1972; Pullman, Coubeils & others, 1972; Katz, Heller & Jacobson, 1973; Weintaub & Hopfinger, 1973) indicates that dopamine tends to reside in three conformational modes representing nearly equivalent energy minima. While the extended conformation (anti) is slightly preferred to the folded conformations (gauche), the energy barrier to rotation between them is not large and therefore it is difficult to conclude which conformer is preferred at the receptor site. On the other hand, studies employing conformationally restricted analogues indicate that the preferred interaction involves a conformer resembling the extended form of dopamine. For example, apomorphine (Colpaert, Van Bever & Leysen, 1976) and certain aminotetrahydronaphthalenes (ATN) such as 5,6-diOHATN and 6,7-diOHATN (Cannon, 1975; Woodruff, Watling & others, 1977) possess significant dopaminergic activity in both central and peripheral models, while analogues resembling the folded form such as the tetrahydroisoquinoline, I (Fig. 1), (Miller, Horn & others, 1974; Volkman, Kohli & others, 1977) and *cis*-(3,4-methylenedioxyphenyl)-cyclopropylamine, III, (Costall, Naylor & Pinder, 1974) are reported to be inactive or to exhibit greatly reduced activity (Miller & others, 1974).

We now report the preliminary biological evaluation of (±)-(E)-2-(3,4-dihyroxyphenyl)cyclopropylamine hydrochloride (ASL-7003). This analogue which is conformationally restricted to resemble the extended form of dopamine, was designed to obtain enhanced selectivity for the dopamine receptor. The cyclopropane system was chosen to rigidly extend the side chain of dopamine because it introduces minimal, potentially adverse steric effects. Examination of molecular models shows tht ASL-7003 is nearly superimposable with the extended form of dopamine and with the reputed pharmacophores of apomorphine and 6,7-diOHATN. Finally, this system offers an opportunity to explore the importance of configurational isomerism for interaction with the dopamine receptor, an area having received little attention.

Chemistry. The synthesis of ASL-7003 hydrochloride salt was accomplished by diazomethane cyclopropanation [$Pd(OAc)_2$] of a catechol-protected cinnamate derivative. The product was obtained by a Curtius conversion to the amine followed by deblocking to the catechol. Experimental details will be published elsewhere. Crystallization from ethanol occurs as an alcoholate have *ca.* 6 mol % ethanol. Physical constants are as follows: m.p. 186–187°; EIMS, $M^{+\cdot}=165$; Anal. ($C_9H_{12}NO_2Cl$) C, H and N; nmr (D_2O) δ 6·7 (m, 3H, ArH), 2·9 (m, 1H, cyclopropyl), 2·4 (m, 1H, cyclopropyl) 1·4 (m, 2H, cyclopropyl).

Pharmacology. Dopaminergic activity was evaluated in the canine renal blood flow model (Goldberg, Sonneville

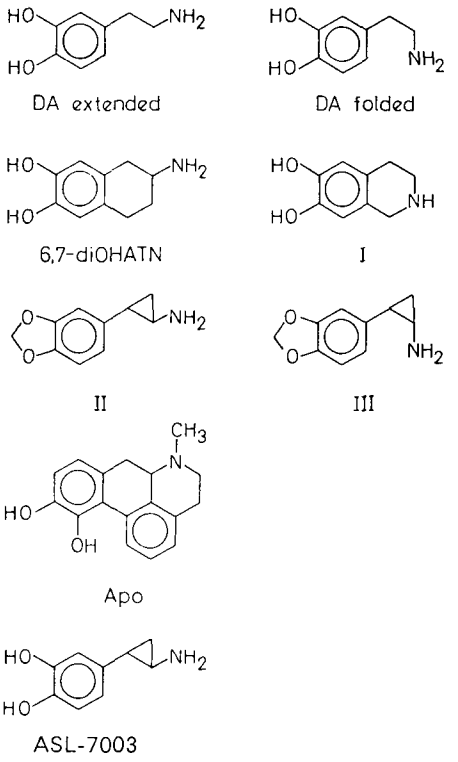

FIG. 1. Structures of the extended and folded forms of dopamine (DA) and their corresponding conformationally restricted analogues. The α- and β-rotameric extremes of both the extended and folded form of dopamine are not considered here (Cannon, 1975). Apo: Apomorphine.

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& McNay, 1968). With appropriate blocking agents, this preparation can be used to distinguish dopaminergic from α- or β-adrenergic activity, or both, after intra-arterial administration of test substances. In contrast to lower doses of dopamine (1–10 μg) which produce vasodilatation, ASL-7003 (1–1000 μg) produced only vasoconstriction. Furthermore, after phenoxybenzamine treatment, dopamine at 1–500 μg continued to produce vasodilatation whereas ASL-7003 (1–1000 μg) produced attenuated vasoconstriction. To examine the effect of the ethanol present as a solvent of crystallization, appropriate ethanol concentrations were studied alone and in combination with dopamine. Since ethanol produced only increases in blood flow and did not decrease the response to dopamine, it is unlikely that its presence in ASL-7003 could have caused the vasoconstriction observed with this compound. Finally, the ASL-7003 racemate was tested for antagonism of dopamine (10 μg) after phenoxybenzamine treatment and was found to be wthout effect at doses of 10, 50 and 100 μg.

To pursue the apparent α-agonist properties observed in the renal preparation ASL-7003 was further studied in the isolated canine hindlimb preparation perfused at constant blood flow. Since flow was held constant, drug-induced changes in perfusion pressure directly reflected changes in hindlimb vascular resistance. The potency of ASL-7003 was compared with noradrenaline in the same preparation before and after propranolol treatment (0·2 mg kg^{-1}, i.v.). Noradrenaline and ASL-7003 produced dose-dependent increases in perfusion pressure which were unaffected by propranolol. Although the linear portion of the dose-response curve for ASL-7003 was approximately parallel to that for noradrenaline, ASL-7003 ($2{\cdot}5 + 10^{-8}$ mol kg^{-1}) was approximately 35 times less potent than noradrenaline ($6{\cdot}45 \times 10^{-10}$ mol kg^{-1}) in producing a 50 mmHg increase in perfusion pressure. Finally, phentolamine (2·0 mg kg^{-1}) competitively blocked the effects of both noradrenaline and ASL-7003 as indicated by parallel shifts of their dose-response curves to the right.

Taken together, these results show that ASL-7003 possesses α-agonist properties while lacking dopaminergic activity in the canine renal blood flow model. This finding was unexpected due to the close structural similarity of ASL-7003 to the extended form of dopamine. However, if an explanation of the lack of dopaminergic activity by structural comparisons is attempted it is found that unlike 6,7-diOHATN and apomorphine whose overall molecule shapes approach planarity, ASL-7003 has the methylene group of the cyclopropyl system residing perpendicular to and therefore out of the general molecular plane. This protrusion may in fact prevent effective drug-receptor interaction in this peripheral dopaminergic model. Alternatively, it could be pointed out that although ASL-7003 has its ethylamine side chain locked in a *trans*-conformation, it is slightly twisted from the fully extended, 'ideal' conformation of dopamine. However, as suggested by Carlström (1975) for an analogous phenylcyclopropylamine system, this deflection of the chain could be counteracted by a larger τ_1 angle [the τ_1 angle has rotational freedom (Pauling, 1973)] resulting in the amino nitrogen atom assuming a position almost identical with respect to the aromatic ring to that of an 'ideal' phenethylamine.

In consideration of the above, it is difficult to conclude whether these steric parameters or others yet unrecognized are responsible for the inactivity of this compound in the canine renal blood flow model. Interestingly, the 3,4-methylenedioxy derivative of tranylcypromine (II), a compound structurally similar to ASL-7003, has been found to exhibit central dopaminergic activity by direct intracerebral administration (Costall & others, 1974). This apparent anomally may be explained by the reported differences in structural requirements for activation of central *vs* peripheral dopaminergic receptors (Goldberg, Volkman & Kohli, 1977). To date, ASL-7003 has not been evaluated in models of central dopaminergic activity.

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The antagonism of the analgesic effect of dipyrone by L-dopa and its relation to brain amine concentrations

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Brain biogenic amines, particularly 5-HT and noradrenaline, have a major role in the mediation of the analgesic response (Montoya & Bardisa, 1970; Jauhari & Bapat, 1971). Most studies correlating analgesia and brain monoamines have used narcotic analgesics (see Sparkes & Spencer, 1971). The non-addictive antipyretic analgesics, however, have received less attention. Goerlitz & Frey (1972) reported that no significant change in the analgesic effect of amino-antipyrine was brought about by prior treatment with antagonists of 5-HT or catecholamines. However, Paalzow (1973), suggested that salicylates exerted at least part of their analgesic action by interference with catecholaminergic neurons.

In the present study, the oral median analgesic dose (AD50) of dipyrone (Novalgin, Hoechst), determined in Swiss albino mice using the hot plate technique (Woolfe & Macdonald, 1944) at 55° (±0·02°), was found to be 90·0 mg kg^{-1} orally, with fiducial limits 62·1–130·5 at $P = 0{\cdot}05$.

The hot plate reaction time (HPRT) was determined in 5 groups of animals, 30 mice each, given saline, dipyrone, 90·0, and 450 mg kg^{-1} by mouth, L-dopa, 100 mg kg^{-1} (i.p.), and dipyrone (90 mg kg^{-1}) simultaneously with L-dopa (100 mg kg^{-1}) at 30, 60, and 90 min after initial drug administration. The normal HPRT was taken as the mean of two determinations, 30 min and immediately before treatment.

To estimate the brain concentrations of 5-HT and noradrenaline, five groups of 24 mice were treated in the same way as for HPRT determination. The animals were killed 1 h after treatment, the whole brains of 4 mice were pooled and amine estimations made spectrophotofluorometrically using a modification of the method of Mead & Finger (1961). Chromatographic separation of the catecholamines ensured that dopamine did not interfere with the noradrenaline estimation.

L-Dopa, given alone, induced a transient increase in the HPRT 30 min after injection (Table 1). Dipyrone induced marked analgesia, which was maintained over 90 min. When dipyrone was given simultaneously with L-dopa, the combination induced a significant increase in the HPRT, which was maintained for 30 min.

Although dipyrone, 90 mg kg^{-1}, induced a 17% decrease in the concentration of 5-HT in the brain and a 28% decrease in that of noradrenaline the ratio of 5-HT: noradrenaline was significantly increased by about 16% from normal. The higher dose of dipyrone (450 mg kg^{-1}) induced a significant increase in 5-HT, without affecting the noradrenaline concentration of the brain. Accordingly, the ratio of 5-HT: noradrenaline was elevated by 23% above the control.

Treatment with L-dopa alone caused a marked increase in the noradrenaline concentration of the brain to the extent of 150% without significant change in the 5-HT concentration. The ratio of 5-HT: noradrenaline was significantly reduced by about 34%. The combination of dipyrone with L-dopa had a similar effect to L-dopa alone. Thus the noradrenaline concentration was significantly raised by 52% and though the 5-HT concentration was also raised by 30%, the value was not statistically significant. The ratio of 5-HT: noradrenaline was not significantly affected by the combined treatment.

The observed analgesia of dipyrone thus correlates more with its effect on the brain ratio of 5-HT: noradrenaline, rather than with its effect on the brain concentration of either amine. The higher dose of dipyrone, caused a greater rise in 5-HT concentration and consequently in the 5-HT: noradrenaline ratio. The concomitant administration of L-dopa lowered this ratio, and subsequently returned the sensitivity of the animals to the nociceptive thermal stimulus to normal.

The relation between brain amines and analgesia is controversial. Morphine analgesia has been shown to be antagonized by inhibitors of catecholamine biosynthesis (Verri, Graff & Carrado, 1967) or by depletors of 5-HT (Major & Pleuvry, 1971). The participation of 5-HT in the central mediation of morphine analgesia was shown by Goerlitz & Frey (1972), but this was disputed by Buxbaum, Yarbrough & Carter (1973) who furthermore reported potentiation of morphine analgesia by α-methyltyrosine. Morphine analgesia was shown to be dependent on the 5-HT: dopamine ratio (Pleuvry & Tobias, 1971) or on the 5-HT: noradrenaline ratio (Sparkes & Spencer, 1971). An increase in 5-HT would therefore promote analgesia, while a decrease would tend to antagonize the antinociceptive effect.

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