J. Pharm. Pharmacol. 1986, 38: 689–691
Communicated March 14, 1986

Effect of urapidil on rat brain catecholamine synthesis

T. A. PUGSLEY*, S. MYERS, *Dept. Pharmacology, Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, Michigan 48105, USA*

The effects of urapidil, a clinically effective antihypertensive drug, on the in-vivo rate of synthesis of rat brain noradrenaline (NA) and dopamine (DA) was determined. A significant dose-dependent increase in dopa concentration by urapidil after dopa decarboxylase inhibition by NSD 1015 was observed in brain stem (3–30 mg kg^{-1} i.p.) and striatum (10–30 mg kg^{-1} i.p.), indicating increased NA and DA turnover, respectively, most probably a result of central blockade of brainstem α_1-adrenergic and striatal DA receptors. These results indicate that urapidil may possibly exert its central hypotensive action in part by a reduced influence on brain stem NA, thus reducing central sympathetic outflow.

The turnover of noradrenaline (NA) in brain and heart is increased by α_1-adrenoceptor blocking agents like phenoxybenzamine, aceperone and prazosin (Dairman et al 1968; Andén et al 1967; Braestrup & Nielson 1976; Fuller et al 1978) and decreased by α_2-adrenoceptor agonists like clonidine (Andén et al 1970). Urapidil is a phenylpiperazine-substituted uracil derivative which lowers blood pressure in man, in both spontaneously and renal hypertensive rats, in normotensive cats (Schoetensack et al 1983) and in rats (van Zwieten et al 1985a). Peripheral α_1-adrenoceptor antagonism probably explains a major part of its accepted antihypertensive effect, but a centrally mediated reduction of peripheral sympathetic flow has also been suggested to play a contributing role (Schoetensack et al 1983). Presynaptic activity at the level of the postganglionic α_2-adrenoceptor may also be of importance in the hypotensive action of urapidil (Eltze 1979). The latter has been questioned by recent studies (Kellar et al 1984; Shebuski & Zimmerman 1985; van Zwieten et al 1985a). Binding studies indicate that urapidil has 90-times greater affinity for α_1- than for α_2-adrenoceptors, with moderate affinity for dopamine DA_2 receptors (Kellar et al 1984). The present study investigates the effects of hypotensive doses of urapidil on the in-vivo rate of the synthesis of brain catecholamines, as a means of further defining its central mechanism of action.

Methods

Male Long Evans rats (200–250 g) from Blue Spruce Farms (Altamont, NY) were used. Synthesis of brain catecholamines was estimated by measuring the accumulation of L-3,4-dihydroxyphenylalanine (dopa) after inhibition of dopa decarboxylase with 100 mg kg^{-1} i.p., of NSD 1015 (3-hydroxybenzylhydrazine; Carlsson et al 1977). The NSD 1015 was administered 30 min after i.p. administration of urapidil (3–30 mg kg^{-1}) or saline. The animals were decapitated 30 min after the NSD 1015. The brain was quickly removed and placed on an ice-cooled plate for dissection of corpus striatum and brain stem minus cerebellum. The brain areas were stored at −70 °C before being assayed.

The brain tissues were homogenized on ice in 0·4 ml of 0·1 M $HClO_4$ containing 200 pM dihydroxybenzylamine as the internal standard. The sonicator tip was washed with 0·1 ml of 0·1 M $HClO_4$ and the wash added to the tissue suspension. Protein was precipitated by centrifugation, and the supernatant decanted into a second set of tubes containing 20 mg of acid-washed alumina. Catechols were adsorbed to the alumina by adding 1·0 ml of 0·5 M Tris-HCl buffer, pH 8·6, and gently agitating for 20 min. The supernatant was aspirated by vacuum, and the alumina pellet was washed three times with 1·0 ml of 0·005 M Tris, pH 8·2, to remove any trapped contaminants. To elute the catechols from the alumina, 0·2 ml of 0·4 M $HClO_4$ was added, the samples were vortexed for 15 s, then centrifuged for 1·0 min. The supernatant was transferred to another set of tubes and retained on ice. An aliquot (5 µl) of the eluate was injected onto a BAS liquid chromatography system (Bioanalytical Systems) fitted with a Perkin Elmer 3 µm ODS C_{18} Column (10 mm × 4·6 mm), a TL5 electrochemical detector cell and an LC-4B controller set at an applied potential of 0·7 V vs an Ag/AgCl reference electrode. The mobile phase was 0·1 M citric acid, 0·06% diethylamine, 0·05 mM EDTA, 0·725 mM sodium octyl sulphate, 7% acetonitrile (pH 3·0). The flow rate was adjusted to 1·4 ml min^{-1}. Standard tests for detection linearity, retention times, and resolution were done using stock solutions of the biogenic amines of interest in 0·4 M $HClO_4$. Quantitation of biogenic amine content was done by integration of peak areas using a Hewlett Packard integrator (Model 3390A).

Results

The effects of i.p. administration of urapidil on dopa accumulation in rat striatum and brain stem after inhibition of dopa decarboxylase are shown in Table 1. In both tissues, urapidil caused a dose-dependent increase in dopa accumulation. A significant increase in dopa concentrations by urapidil was observed in the brain stem at 3, 10 and 30 mg kg^{-1} and in the striatum at

* Correspondence.

Table 1. Estimation of the effect of urapidil on rat brain catecholamine synthesis. Synthesis was estimated by measuring the accumulation of dopa after dopa decarboxylase inhibition by NSD 1015 (100 mg kg^{-1} i.p.) 30 min after administration of urapidil or saline. The animals were decapitated 30 min after the NSD 1015 injection. Each value is mean of 5 animals per group. The numbers in parentheses are percent increases of dopa versus control.

Treatment	Dose (mg kg^{-1} i.p.)	Dopa accumulation (μg g^{-1} ± s.e.m.) Striatum	Brain stem
Control	—	1·01 ± 0·05	0·113 ± 0·009
Urapidil	3	1·19 ± 0·11 (118)	0·143 ± 0·012* (126)
Urapidil	10	1·26 ± 0·09* (125)	0·156 ± 0·010* (138)
Urapidil	30	1·45 ± 0·16* (144)	0·180 ± 0·020** (160)

** $P < 0·01$; * $P < 0·05$ versus control-treated group.

10 and 30 mg kg^{-1} compared with the vehicle-treated control group.

Discussion

The ability of urapidil to increase the accumulation of dopa after inhibition of dopa decarboxylase in the rat brain stem, an NA-enriched area, supports the idea that urapidil blocks central α_1-adrenoceptors. Urapidil has previously been shown to block peripheral α_1-adrenoceptors (Schoetensack et al 1983) and exhibit moderate affinity for rat brain α_1-adrenoceptors (Kellar et al 1984). An increase in NA turnover as a compensatory response to a block of α_1-adrenoceptors has been reported for phenoxybenzamine, phentolamine, aceperone and prazosin (Andén et al 1967; Dairman et al 1968; Braestrup & Nielsen 1976; Fuller et al 1978).

In addition, the accumulation of dopa in rat striatum, a mainly dopaminergically innervated brain area, indicates that urapidil at higher doses is also capable of accelerating the rate of DA synthesis. The mechanism of this effect is unclear. The increase in DA turnover is unlikely to be secondary to central α_1-adrenoceptor blockade as an agent such as prazosin, which is a selective α_1-antagonist, decreases (Fuller et al 1978) rather than increases DA turnover as does urapidil. Urapidil exhibits some affinity for DA_2 receptors (Kellar et al 1984). Thus, one explanation is that urapidil is directly blocking postsynaptic DA_2 receptors, resulting in a compensatory increase in the rate of DA synthesis. An increase in DA synthesis and turnover as a compensatory response to a block of DA receptors has been well documented with antipsychotic agents such as haloperidol and chlorpromazine (Carlsson et al 1977).

Evidence indicates that urapidil has two sites of antihypertensive action, both involving the sympathetic nervous system. The first consists of a blockade of peripheral α_1-adrenoceptors and the other appears to be a centrally mediated effect by an interaction with brain stem structures to suppress sympathetic flow (Schoetensack et al 1983; Kellar et al 1984; Shebuski & Zimmerman 1985; van Zwieten et al 1985a). The present neurochemical findings that, as with known α_1-adrenoceptor blockers, hypotensive doses of urapidil accelerate rat brain stem NA turnover supports the above findings that urapidil has a central component to its blood pressure-lowering effects. The possible DA_2 receptor blockade by urapidil is probably not important for its hypotensive action since a selective and potent DA receptor antagonist such as spiroperidol does not influence the blood pressure of rats (Trolin 1975). In man the hypotensive action of urapidil is not associated with reflex tachycardia (Schoetensack et al 1983). In this regard Shebuski & Zimmerman (1985) have shown that urapidil, and to a lesser extent prazosin, when administered intracisternally, suppresses the baroreceptor-induced reflex tachycardia in anaesthetized dogs. They postulated that the effect of these agents may be due to blockade of central α_1-adrenoceptors which are involved in the increased sympathetic outflow exerted by baroreceptor inhibition. The α_1-adrenoceptor antagonists, prazosin and WB-4101 (2-[*N*-(2,6-dimethoxyphenoxyethyl)]aminomethyl-1,4-benzodioxane), reduce sympathetic outflow in baroreceptor-denervated cats (McCall & Humphrey 1981) and nicergoline, an α_1-antagonist, reduces sympathetic outflow by a central action in dogs (Huchet et al 1981). This evidence, together with the present findings, indicating that urapidil accelerates brain stem NA turnover in-vivo suggests that the central sympathoinhibitory effect produced by these agents is most likely associated at least in part with central α_1-adrenoceptor blockade. However, the effect of α-adrenoceptor drugs and their control of central vasomotor activity is complex (Bousquet & Schwartz 1983). A study by van Zwieten et al (1985b) with urapidil underlines this complexity further. They found that prior treatment of the vertebral artery of chloralose-anaesthetized cats with antagonists of α_1- and α_2-adrenoceptors, and H_1- and H_2-histamine, dopamine, 5-HT, and opiate receptors, did not antagonize the central hypotensive action of urapidil subsequently given. It was concluded that the central mode of action of urapidil remains unexplained. Notwithstanding the latter study, the new information presented here that the α_1-antagonist urapidil, at hypotensive doses, causes a dose-dependent increase in brain stem NA turnover indicates the possibility that urapidil might partially exert its central antihypertensive action by a reduced influence of central NA, due to a blockade of central α_1-adrenoceptors; it is also known that α_1-adrenoceptor agonists, given into the hypothalamus, can cause hypertension (Phillippu et al 1971).

REFERENCES

Andén, N. E., Corrodi, H., Fuxe, K., Hökfelt, T. (1967) Eur. J. Pharmacol. 8: 59–64

Andén, N. W., Corrodi, A., Fuxe, K., Hökfelt, B., Hökfelt, T., Rydin, C., Svensson, T. (1970) Life Sci. 9(I): 513–523

Bousquet, P., Schwartz, J. (1983) Biochem. Pharmacol. 9: 1459–1465

Braestrup, C., Nielsen, M. (1976) J. Pharmacol. Exp. Ther. 198: 595–608

Carlsson, A., Wehr, W., Linqvist, M. (1977) J. Neural Transm. 40: 99–113

Dairman, W., Gordon, R., Spector, S., Sjordsma, A., Undenfriend, S. (1968) Mol. Pharmacol. 4: 457–464

Eltze, M. (1979) Eur. J. Pharmacol. 59: 1–9

Fuller, R. W., Snoddy, H. D., Perry, K. W. (1978) Arch. Int. Pharmacodyn. 231: 30–41

Huchet, A., Mouillé, P., Chelly, J., Lucet, B., Doursout, M., Lechat, P., Schmitt, H. (1981) J. Cardiovasc. Pharmacol. 3: 677–691

Kellar, K. J., Quest, J. A., Spera, A. C., Buller, A., Conforti, A., Dias Souza, J., Gillis, R. D. (1984) Am. J. Med. 77: 87–95

McCall, R. B., Humphrey, S. J. (1981) J. Auton. Nerv. Syst. 3: 9–23

Phillippu, A., Przuntek, H., Heyd, G., Burger, A. (1971) Eur. J. Pharmacol. 15: 200–208

Schoetensack, W., Bruckschen, E. G., Zeeh, K. (1983) in: Scriabine, A. (ed.) New Drugs Annual, Cardiovascular Drugs. Raven Press, New York, pp 19–48

Shebuski, R. J., Zimmerman, B. G. (1985) J. Pharmacol. Exp. Ther. 234: 456–462

Trolin, G. G. (1975) Acta Physiol. Scand. Suppl. 430: 1–41

van Zwieten, P. A., De Jonge, A., Wilffert, B., Timmermans, P. B. M. W. M., Beckeringh, J. J., Thoolen, M. J. M. C. (1985a) Arch. Int. Pharmacodyn. 276: 180–201

van Zwieten, P. A., Mathy, M. J., Thoolen, M. J. M. C. (1985b) J. Pharm. Pharmacol. 37: 810–811

J. Pharm. Pharmacol. 1986, 38: 691–694
Communicated March 6, 1986

Dopaminergic metabolism in various rat brain areas after L-dopa loading

G. El Gemayel, J. H. Trouvin*, M. Prioux-Guyonneau, C. Jacquot, Y. Cohen, *Laboratoire de Pharmacologie, UA CNRS 594, Faculté de Pharmacie, Chatenay-Malabry, F-92290 France*

The time course of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxytyramine (3-MT) and 3-methoxytyrosine (3-*O*-Medopa) concentrations in rat brain after treatment with L-dopa + benserazide has been investigated in the striatum, hypothalamus, hippocampus and cerebellum. These areas were selected for their different dopaminergic activities. After L-dopa loading, DA, DOPAC and HVA were increased in all the structures, but the largest increases were in those tissues with the less dopaminergic activity, while 3-MT increased in the hypothalmus, hippocampus and cerebellum but was lowered in striatum. 3-*O*-Medopa, which is the direct product of the *O*-methylation of L-dopa, did not show any specific distribution. The data provide evidence that the striatum, by feed-back mechanisms and specific enzymatic activity, is able to ensure a better regulation of dopaminergic activity than the other structures, thereby overcoming excess L-dopa.

Lammers & Van Rossum (1968) reported a 'bizarre social behaviour' which occurs after treating male rats with the combination L-dopa and benserazide. Some of the components of this behavioural syndrome could be classed as emotional aggression which is known to have a complex biochemical basis (Reis 1974).

We have previously studied the comportmental effects of such a treatment (El Gemayel et al 1986) which were suggested to be related to different perturbations of the monoamine metabolism after L-dopa loading (Deakin & Dashwood 1981).

The biochemical study of the effects of treatment with L-dopa and benserazide was aimed at elucidating the mechanism of action of L-dopa in the treatment of Parkinson's disease (Bartholini et al 1971). In those studies, fluorimetric and radioactive methods were used to quantify endogenous catecholamine levels (Calne et al 1969). Many explanations were proposed to elucidate this mechanism and the biochemical and comportmental disorders caused by the L-dopa therapy (Hefti et al 1981), however this field has remained little known until recently (Ponzio et al 1984).

We earlier used a chromatographic method with electrochemical detection to study the striatal metabolism of dopamine after L-dopa loading and we found that the simultaneous evaluation of the time course of dopamine (DA), 3,4-dihydroxyphenylacetic acid DOPAC, homovanillic acid (HVA) and 3-methoxytyramine (3-MT) levels over 9 h in this dopaminergic structure showed that there was a dramatic increase in the concentrations of these compounds and then they slowly disappeared. Furthermore, 3-methoxytyrosine (3-*O*-Medopa), an unusual metabolite, was detected in very high concentration (El Gemayel et al 1986). We therefore have undertaken a further study of the evolution of the levels of these five compounds in four brain areas having different dopaminergic innervation. Striatum was selected as the main dopaminergic area, the hypothalamus for its numerous neurotransmitter interactions, the hippocampus as a minor dopaminergic region and the cerebellum as an area completely deprived of DA terminals and perikarya.

Materials and methods

Eighty male Sprague-Dawley rats (Charles Rivers, France) (225–250 g) housed under standard conditions were used. Treatments were randomly attributed according to a 'balanced lattice design' (Cochran & Cox 1957). Treated animals were injected with L-dopa

* Correspondence.