# The effect of prolonged clonidine administration on catecholamine metabolism in the rat brain

ANTHONY J. DRAPER, DAVID GRIMES, PETER H. REDFERN*, *Pharmacology Group, School of Pharmacy and Pharmacology, University of Bath, Bath, Avon, U.K.*

Acute doses of the antihypertensive drug clonidine cause a decrease in the turnover of central catecholamines (Laverty & Taylor, 1969, Andén, Corrodi & others, 1970), although the extent of the decrease appears widely variable. We have examined the effects of more prolonged clonidine administration on both blood pressure and central catecholamine turnover, in normotensive and two types of hypertensive rat, namely renal clip/DOCA rats, and New Zealand spontaneously hypertensive rats (SH-rats).

Male rats of the CFY strain were used, normotensive animals at 200–250 g, while hypertensive animals were used 4 to 5 weeks after surgery on 70–90 g animals, made hypertensive by a modification of the method of Finch & Leach (1970), in which the left renal artery was occluded with a silver clip, and a 25 mg pellet of deoxycorticosterone acetate was implanted subcutaneously. These animals had free access to 1 % saline; increases in mean systolic blood pressure of 70 mmHg (219 ± 5 mmHg, mean ± s.e.m., n = 20) over that of control animals (144 ± 2 mmHg, n = 20) resulted. SH-rats were from a colony of the New Zealand strain raised in our own laboratories, with blood pressures of 208 ± 4 mmHg (n = 19). All blood pressures were measured in the conscious restrained animal by the tail cuff method before dosing and on the sixth day. Clonidine hydrochloride in 0·9 % saline was given intraperitoneally twice daily at 9.00 and 17.00 h for 7 days, controls were given saline. Statistical significances were determined using Student's *t*-test.

To estimate the effect of clonidine on the concentration of noradrenaline and dopamine in the brain, animals were killed 2 h after the last morning dose of clonidine, the brains dissected into regions (Glowinski & Iversen, 1966) and the catecholamines extracted according to Anton & Sayre (1962) and then assayed fluorimetrically (Shellenberger & Gordon, 1971).

The turnover of the two amines was estimated in whole brains by measuring the rate of decline of the amine concentrations after synthesis inhibition with α-methyl-*p*-tyrosine methyl ester hydrochloride (α-MT) (250 mg $kg^{-1}$, i.p.) (Brodie, Costa & others, 1966). Concentrations of the amines were measured at time 0 and 4 h after α-MT, previous experiments having shown that the decline was linear over this period. Turnover rates were compared by log-linear regression analysis of the results and comparison of the resultant slopes by *t*-test. In clonidine-treated animals, α-MT was administered 2 h after the last morning dose, and the animals killed 4 h later.

* Correspondence.

Drugs used were: clonidine hydrochloride (Boehringer-Ingelheim), α-methyl-*p*-tyrosine methyl ester hydrochloride, (Sigma) and deoxycorticosterone acetate (Organon).

In normotensive animals clonidine (50 or 100 μg $kg^{-1}$, i.p.) produced significant falls in blood pressure (12·1 and 20·6 %, $P < 0.001$ for both), but produced no significant changes in the concentration of the amines in brain regions (cortex, midbrain, hypothalamus, and medulla-pons) or whole brain. However in the renal/DOCA hypertensive animal the same doses of clonidine not only produced similar significant changes in blood pressure (15·1 and 20·9 % for the low and high doses of clonidine, $P < 0.01$) but also significant increases in the concentrations of noradrenaline (Fig. 1), in certain brain areas no consistent changes were found in dopamine concentration.

The rate of decline of noradrenaline concentration after synthesis inhibition with α-MT was measured in whole brains of normotensive and renal/DOCA hypertensive rats. In the latter, the rate of decline was significantly decreased by 30·1 % of the control value (Table 1). Similar results were obtained for the rate of decline of dopamine concentrations (Table 1).

When these experiments were repeated using clonidine-treated animals it was found that in all animals clonidine produced significant and dose related decreases in the turnover of both amines (as well as significant falls in blood pressure (Table 2).

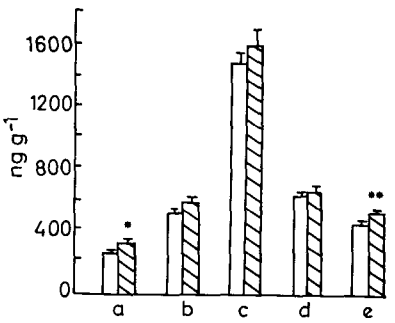


FIG. 1. The effect of clonidine 100 μg $kg^{-1}$ (i.p.) twice daily for 7 days on the concentrations of noradrenaline in rat brain (ng $g^{-1}$ tissue). Open columns represent control values, hatched columns represent clonidine treated values. Values are the mean of 10 samples (±s.e.m.) * $P < 0.05$, ** $P < 0.01$. a-Cortex, b-midbrain, c-hypothalamus, d-medulla pons, e—total brain.

Table 1. *Rate of decline of catecholamine concentrations in the brains of normotensive and renal/DOCA hypertensive rats after synthesis inhibition.* Blood pressures were measured, and concentrations of noradrenaline (NA) and dopamine (DA) estimated after synthesis inhibition by α-methyl-*p*-tyrosine (250 mg $kg^{-1}$, i.p.). Rates were compared by comparison of slopes of graphs.

| | No. of animals | Mean blood pressure mm Hg (±s.e.m.) | Rates of decline NA | DA |
|---|---|---|---|---|
| Normotensive rats | 10 | 144 ± 2 | 0·07662 | 0·1357 |
| Renal/DOCA hypertensive rats | 10 | 219 ± 5 | 0·05359 | 0·0952 |
| % decrease in the rate of decline | — | — | 30·1% | 29·6% |
| Significance | — | $P<0.001$ | $P<0.01$ | $P<0.01$ |

Table 2. *The effect of clonidine on blood pressure, and on the rate of decline of noradrenaline (NA) and dopamine (DA) in the brains of normotensive and hypertensive rats.* Clonidine (50 or 100 μg $kg^{-1}$, i.p.) was given twice daily for 7 days. Blood pressures were measured the day before killing, and the rate of decline of NA and DA estimated after synthesis inhibition by α-methyl-*p*-tyrosine. Results are expressed as percentage decrease from corresponding untreated normotensive and untreated hypertensive animals.

| Drug and dose μg $kg^{-1}$ | n | % decrease in b.p. | % decrease in rate of decline NA | DA |
|---|---|---|---|---|
| Normotensives | | | | |
| Clonidine 50 | 10 | 12·1%*** | 10·7% | 5%† |
| Clonidine 100 | 10 | 20·6%*** | 48·7%** | 5% |
| Renal/DOCA hypertensive | | | | |
| Clonidine 50 | 6 | 15·1%** | 15·9% | 2·2% |
| Clonidine 100 | 7 | 20·9%** | 40·5%* | 12·1% |
| New Zealand S.H. | | | | |
| Clonidine 100 | 8 | 26·0%*** | 66·4%*** | 19·8%*** |

* = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$; † = increase.

The rate of decline of amine concentration after synthesis inhibition is presumably related to the rate at which the amines are released from their binding sites within the nerve endings, and subsequently destroyed and/or removed from the brain.

We have demonstrated that this 'turnover' rate of noradrenaline is significantly less in hypertensive than in normotensive animals, confirming the results of Nakamura, Gerold & Thoenen, (1971) and Ito, Tanaka & Omac, (1975). While noradrenaline can be shown to be either depressor or pressor depending on the area of the brain to which it is applied, (Gagnon & Melville, 1968; Phillipu, Przuntek & others, 1971), it is now widely believed to have a predominantly inhibiting influence on blood pressure (De Jong, 1974; Struykier Boudier, Smeets & others, 1975) a hypothesis with which our results are compatible.

This inhibitory influence of noradrenaline on blood pressure is thought to be mediated via α-adrenoceptors (Haeusler, 1974; De Jong 1974) and it is also believed that clonidine's action on these same α-receptors accounts for the drug's antihypertensive action (Andén & others, 1970; Van Zwieten, 1973; Kobinger & Pichler, 1974). Such an action might be brought about either by a post-synaptic action in which clonidine replaced noradrenaline as an α-adrenoceptor agonist, or by an action on pre-synaptic α-adrenoceptors.

For a presynaptic action to be compatible with the hypothesized inhibitory role of noradrenaline, it must be assumed that clonidine is acting presynaptically as an antagonist to noradrenaline at α-adrenoceptors on the pre-synaptic membrane (Stjarne, 1975) and allowing more noradrenaline to be released in response to stimulation of the presynaptic neuron. In other words the fall in blood pressure produced by clonidine would be associated with an increased turnover of noradrenaline. But we have found that the rate of decline of noradrenaline following synthesis inhibition to be significantly slowed by clonidine given over seven days at 100 μg $kg^{-1}$ (a decrease also occurred at 50 μg $kg^{-1}$ but was not statistically significant). This occurred in normotensive rats, and in hypertensive rats in which noradrenaline turnover was already significantly reduced.

Using another functional model, Andén, Grabowska & Strombom (1976) have suggested that the metabolic and functional effects of clonidine are unrelated, being mediated by pre- and post-synaptic α-adrenoceptors respectively. While we cannot rule out the possibility that a similar separation occurs in the areas of the brain responsible for blood pressure control, there seems no reason to suppose that bombardment of post-synaptic α-receptors should not be responsible for both the recorded fall in blood pressure and the decreased turnover of noradrenaline, by a feedback homeostatic mechanism.

Thus our results using prolonged clonidine administration are compatible with the hypothesis that the antihypertensive effect of clonidine is brought about by an agonist action on post-synaptic receptors.

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REFERENCES

ANDÉN, N.-E., CORRODI, H., FUXE, K., HOKFELT, B., HOKFELT, T., RYDIN, C. & SVENSSON, T. (1970). *Life Sci.* **9**, 513–523.
ANDÉN, N.-E., GRABOWSKA, M. & STROMBOM, U. (1976). *Naunyn Schmiedebergs Arch. Pharmac.*, **292**, 43–52.
ANTON, A. H. & SAYRE, D. F. (1962). *J. Pharmac. exp. Ther.*, **138**, 360–375.
BRODIE, B. B., COSTA, E., DLABAC, A., NEFF, N. H. & SMOOKLER, H. H. (1966). *Ibid.*, **154**, 493–498.
DE JONG, W. (1974). *Eur. J. Pharmac.*, **29**, 179–181.
FINCH, L. & LEACH, G. D. H. (1970). *Br. J. Pharmac.*, **39**, 317–324.
GAGNON, D. J. & MELVILLE, K. I. (1968). *Can. J. Physiol. Pharmac.*, **46**, 595–599.
GLOWINSKI, J. & IVERSEN, L. L. (1966). *J. Neurochem.*, **13**, 655–669.
HAEUSLER, G. (1974). *Naunyn Schmiederbergs Arch. Pharmac.*, **285**, 1.
ITO, A., TANAKA, K. & OMAC, T. (1975). *Jap. Heart. J.*, **16**, 575–582.
KOBINGER, W. & PICHLER, L. (1974). *Eur. J. Pharmac.*, **27**, 151–154.
LAVERTY, R. & TAYLOR, K. M. (1969). *Br. J. Pharmac.*, **35**, 253–264.
NAKAMURA, K., GEROLD, M. & THOENEN, H. (1971). *Naunyn Schmiederbergs Arch Pharmac.*, **268**, 125–139.
PHILLIPU, A., PRZUNTEK, H., HEYD, G. & BURGER, A. (1971). *Eur. J. Pharmac.*, **15**, 200–208.
SHELLENBERGER, M. K. & GORDON, J. H. (1971). *Analyt. Biochem.*, **39**, 356–372.
STJANE, L. (1975). *Acta Physiol. Scand.*, **93**, 142–144.
STRUYKER BOUDIER, H., SMEETS, G., BROUWER, G. & VAN ROSSUM, J. M. (1975). *Archs int. Pharmacodyn. Thér.*, **213**, 285–293.
VAN ZWIETEN, P. A. (1973). *J. Pharm. Pharmac.*, **25**, 89–95.

# The influence of mescaline on the flexor reflex of the hind limb of the spinal rat

J. MAJ*, W. PALIDER, A. RAWŁÓW, *Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland*

We have stated previously that serotoninergic agents like L-5-hydroxytryptophan, L-tryptophan, LSD and fenfluramine stimulate the flexor reflex of the spinal rat which is antagonized by the 5-hydroxytryptamine (5-HT) receptor blockers, cyproheptadine, Danitracen (WA-335) and methergoline (Maj, Palider & Baran, 1976b). Mescaline possesses a strong depleting action on the 5-HT of blood platelets, which are regarded as a model of 5-HT neurons (May, Menkens & Westermann, 1969). Also, electrophysiological and biochemical findings show evidence of the influence of mescaline on 5-HT neurons (Tonge & Leonard, 1968; Aghajanian, Foot & Sheard, 1970; Bradshaw, Roberts & Szabadi, 1971; Haigler & Aghajanian, 1972; Tilson & Sparber, 1972; Bevan, Bradshaw & others, 1974). We now report the action of mescaline on the flexor reflex of the spinal rat, and the influence on this action of cyproheptadine and WA-335 (Stone, Wenger & others, 1961; van Riezen, 1972; Engelhardt, 1975; Maj, Baran & others, 1976a). Experiments were on Wistar male rats, 180–270 g, according to the method described previously in which an animal paw is stimulated electrically and contractions of the musculus tibialis anterior recorded (Maj & others, 1976b). Drugs: clomipramine, desipramine, imipramine—all as hydrochlorides and mescaline sulphate were injected into the femoral vein as solution in 0·9% NaCl. Cyproheptadine and WA-335 were used intraperitoneally as suspensions in a 1% aqueous solution of Tween 80.

Mescaline 1·5–10 mg $kg^{-1}$, stimulated the flexor reflex of the spinal rat (Fig. 1). The effect appeared immediately after injection and lasted 30–90 min. As

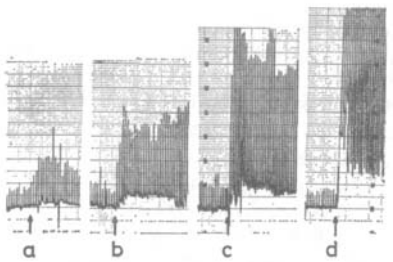


FIG. 1. Showing the effect of mescaline in 0·9% NaCl solution given into the femoral vein a—1·5, b—2, c—3, d—5 mg $kg^{-1}$ on the flexor reflex (m. tibialis anterior) of the hind limb of the spinal rat.

* Correspondence.