Department of Pharmacology, P.F.662,
Medical Faculty,
Belgrade 11 000, Yugoslavia.

DRAGINJA ANDJELKOVIĆ

July 19, 1973

REFERENCES

EGLE, J. L., MEREDITH, J. H. & LITTLE, J. M. (1967). *J. Pharmac. exp. Ther.*, **158**, 399–404.

UMBREIT, W. W., BURRIS, R. H. & STUFFER, J. F. (1957). *Manometric techniques.* Minneapolis: Burgess Publishing Co.

Effects of phenoxybenzamine, aceperone and clonidine on the level of 3-methoxy-4-hydroxyphenylglycol (MOPEG) in rat brain

In the central nervous system, α-adrenoceptor antagonists have been shown to increase noradrenaline turnover, measured either by the increase in [^{14}C]noradrenaline synthesis after intravenous [^{14}C]tyrosine (Dairman, Gordon & others, 1968) or by the disappearance of endogenous noradrenaline after synthesis inhibition (Corrodi, Fuxe & Hökfelt, 1967; Andén, Corrodi & Fuxe, 1972). On the contrary, the α-adrenoceptor agonist, clonidine (Andén, Corrodi & others, 1970), has been shown to decrease central noradrenaline turnover (Andén & others, 1970). In *in vitro* tissue preparations α-adrenoceptor antagonists and agonists have been shown to increase and decrease stimulation-induced noradrenaline overflow respectively, and these results have been interpreted in terms of compensatory changes in noradrenaline release following decreased or increased α-adrenoceptor activity (Farnebo & Hamberger, 1971; Starke & Altmann, 1973; Häggendal, Johansson & others, 1972; Potter, Chubb & others, 1971; Enero, Langer & others, 1972).

To achieve some information about whether the changes in noradrenaline turnover in the central nervous system *in vivo* following α-adrenoceptor active drugs are in any way related to changes in central noradrenaline release, I have studied the effect of the α-adrenoceptor antagonists, aceperone (Janssen, Niemegeers & others, 1967) and phenoxybenzamine, and the α-adrenoceptor agonist, clonidine, on the level of 3-methoxy-4-hydroxyphenylglycol (MOPEG) in the cns of rats. The endogenous level of MOPEG in the cns has not previously been used as a measure of central noradrenaline release, but recent experiments indicate that the level of MOPEG is useful for this purpose (Braestrup, Nielsen & others, in preparation; Walter & Eccleston, 1972, 1973; Korf, Aghajanian & Roth, 1973). The consistent results obtained in the present study further validate the use of this new technique.

Male Wistar rats, about 270 g, kept at room temperature (22°), were injected with one of the following drugs: aceperone (20 mg kg^{-1}), phenoxybenzamine (20 mg kg^{-1}), clonidine (0·5 mg kg^{-1}), protriptyline (10 mg kg^{-1}) or saline (1 ml kg^{-1}). One to 4 h after drug administration, animals were decapitated and total MOPEG, or in some experiments free MOPEG, was estimated in saline controls in parallel with drug-treated animals as described previously (Braestrup, 1973). Brains were homogenized in acetic acid, conjugates were hydrolysed with glusulase, MOPEG was extracted into ethyl acetate and the pentafluoropropionyl derivative was prepared for g.l.c. on an OV-17 column followed by electron capture detection. Analyses of variance followed by *t*-tests of control versus drug-treated animals were used for statistical evaluation. Most values are expressed as per cent of the control with a s.e. value denoting the dispersion of the drug-treated animals only.

Phenoxybenzamine (20 mg kg^{-1}) and aceperone (20 mg kg^{-1}) both caused a signifi-

Table 1. *Effect of time of phenoxybenzamine* (20 mg kg^{-1}), *aceperone* (20 mg kg^{-1}) *and clonidine* (0·5 mg kg^{-1}) *administration on total MOPEG level in the whole rat brain.* Each value is the mean ± s.e. of (n) determinations in per cent of control. Total MOPEG in control animals averaged 82·3 ± 2·2 ng g^{-1} tissue (n = 51).

	1 h	2 h	3 h	4 h
Aceperone	143·5 ± 12·0* (9)	155·4 ± 11·5*** (10)	—	161·3 ± 11·3*** (13)
Phenoxybenzamine	162·0 ± 16·2* (4)	—	—	157·9 ± 8·8*** (4)
Clonidine	78·1 ± 7·6 (4)	72·8 ± 3·7*** (7)	66·4 ± 2·3** (4)	—

* = $P < 0{\cdot}025$; ** = $P < 0{\cdot}005$; *** = $P < 0{\cdot}001$ relative to controls.

cant increase in total MOPEG between 1 and 4 h after administration (Table 1). Clonidine (0·5 mg kg^{-1}) caused a significant decrease in total MOPEG 2 and 3 h after administration; the decrease observed after 1 h was just significant ($P < 0{\cdot}046$).

The increase in MOPEG after administration of α-adrenoceptor blocking drugs and the decrease caused by the α-adrenoceptor stimulant indicate that these drugs respectively increased and decreased the release of noradrenaline in central neurons. Previous studies evaluating central noradrenaline release *in vivo* by the accumulation of normetanephrine after monoamine oxidase inhibitors have shown small and hardly significant effects of phenoxybenzamine and aceperone (Carlsson & Lindqvist, 1963; Scheel-Krüger, 1972). These results, however, are hampered by the complication that monoamine oxidase inhibitors might themselves influence the regulation in the noradrenergic system and thus cover the effect of α-adrenoceptor antagonists.

Apart from increasing noradrenaline release, other possible actions of phenoxybenzamine and aceperone should be considered. 1. Phenoxybenzamine is known to inhibit both neuronal and extraneuronal (Lightman & Iversen, 1969) uptake of noradrenaline in tissue preparations. Inhibition of neuronal uptake is, however, an unlikely explanation for the increase in MOPEG, since the potent inhibitor of neuronal uptake, protriptyline (Carlsson, Corrodi & others, 1969), does not increase MOPEG (90·7% ± 6·7 (4) of control after 1 h, $P > 0{\cdot}37$) (see also Häggendal & others, 1972). Inhibition of extraneuronal uptake by phenoxybenzamine would be expected to result in a decrease in MOPEG (Langer, 1970); on the contrary an increase was observed in the present study. 2. The possibility that phenoxybenzamine and aceperone inhibit the disappearance of MOPEG-SO_4 is not favoured by previous results, showing that the disappearance of MOPEG-SO_4 from the rat brain is unaltered by phenoxybenzamine (Meek & Neff, 1973), and because the level of free MOPEG (13·0 ± 0·8 ng g^{-1} tissue, n = 10) is significantly increased by aceperone (20 mg kg^{-1}, 2 h) (131·2 ± 9·3% n = 7, $P < 0{\cdot}025$). 3. The increased synthesis and turnover of noradrenaline after α-adrenoceptor antagonists might be accompanied by increased overflow-degradation of noradrenaline to MOPEG without a concomitant release of noradrenaline. This possibility cannot be excluded at present.

Concerning the decrease in MOPEG following clonidine, some alternative explanations must be considered.

1. Previous results have indicated that clonidine might inhibit extraneuronal uptake of noradrenaline in the rat isolated heart (Salt, 1972), according to Lightman & Iversen (1969), any catecholamine taken up by extraneuronal uptake is rapidly metabolized, and inhibition of this effect *per se* might decrease MOPEG formation. The increase in total MOPEG observed following the other strong extraneuronal

uptake inhibitor phenoxybenzamine does not favour this hypothesis.

2. The profound hypothermia seen after clonidine (34·2 ± 0·3° (6) after 2 h) compared to saline (37·4 ± 0·3° (4)) does not appear to be responsible for the decrease in MOPEG after clonidine, since the MOPEG decrease persists when body temperature is kept within normal range by increasing the room temperature to 30°. (Total MOPEG averaged 70·6 ± 3·2% (3) of control at 1 h, $P < 0{\cdot}009$.)

3. The disappearance rate of MOPEG-SO_4 does not appear to be increased since free MOPEG (13·0 ± 0·8 ng g^{-1} tissue n = 10) also is decreased, 1 h following clonidine (0·5 mg kg^{-1}) administration (38·8 ± 4·5%, n = 4, of control; $P < 0{\cdot}001$).

In conclusion, the present results indicate that the α-adrenoceptor antagonists phenoxybenzamine and aceperone increase noradrenaline release, while the α-adrenoceptor agonist clonidine decreases noradrenaline release in the cns of rats. The results thus agree with the contention that the activity of noradrenergic neurons is compensatorily regulated following blockade or stimulation of the α-adrenoceptors in the cns of rats (Carlsson & Lindqvist, 1963; Andén, & others, 1970; Farnebo & Hamberger, 1971).

This work was supported by a grant from Det Laegevidenskabelige Forskningsråd, Copenhagen. Skilful technical assistance was given by Mrs. Mette Buhl. The generous gift of clonidine by Boehringer Ingelheim is much appreciated.

Central Laboratory, Sct Hans Hospital, Dept. E,
DK-4000 Roskilde,
Denmark.

C. BRAESTRUP

August 29, 1973

REFERENCES

ANDÉN, N.-E., CORRODI, H., FUXE, K., HÖKFELT, B., HÖKFELT, T., RYDIN, C. & SVENSSON, T. (1970). *Life Sci.*, **9**, 513–523.

ANDÉN, N.-E., CORRODI, H. & FUXE, K. (1972). *J. Pharm. Pharmac.*, **24**, 177–182.

BRAESTRUP, C. (1973). *Analyt. Biochem.*, **55**, 420–431.

CARLSSON, A. & LINDQVIST, M. (1963). *Acta pharmac. tox.*, **20**, 140–144.

CARLSSON, A., CORRODI, H., FUXE, K. & HÖKFELT, T. (1969). *Eur. J. Pharmac.*, **5**, 367–373.

CORRODI, H., FUXE, K. & HÖKFELT, T. (1967). *Life Sci.*, **6**, 767–774.

DAIRMAN, W., GORDON, R., SPECTOR, S., SJOERDSMA, A. & UDENFRIEND, S. (1968). *Mol. Pharmac.*, **4**, 457–464.

ENERO, M., LANGER, S., ROTHLIN, R. & STEFANO, F. (1972). *Br. J. Pharmac.*, **44**, 672–688.

FARNEBO, L.-O. & HAMBERGER, B. (1971). *Acta physiol. scand. suppl.*, **371**, 35–44.

HÄGGENDAL, J., JOHANSSON, B., JONASON, J. & LJUNG, B. (1972). *J. Pharm. Pharmac.*, **24**, 161–164.

JANSSEN, P., NIEMEGEERS, C., SCHELLEKENS, K. & LENAERTS, F. (1967). *Arzneimittel-Forsch*, **17**, 841–854.

KORF, J., AGHAJANIAN, G. & ROTH, R. (1973). *Eur. J. Pharmac.*, **21**, 305–310.

LANGER, S. Z. (1970). *J. Physiol.*, **208**, 515–546.

LIGHTMAN, S. L. & IVERSEN, L. L. (1969). *Br. J. Pharmac.*, **37**, 638–649.

MEEK, J. L. & NEFF, N. H. (1973). *J. Pharmac. exp. Ther.*, **184**, 570–575.

POTTER, W. P. De, CHUBB, I. W., PUT, A. & SCHAEPDRYVER, A. F. De (1971). *Archs int. Pharmacodyn. Thér.*, **193**, 191–197.

SALT, P. J. (1972). *Eur. J. Pharmac.*, **20**, 329–340.

SCHEEL-KRÜGER, J. (1972). *Archs int. Pharmacodyn. Thér.*, **195**, 372–378.

STARKE, K. & ALTMANN, K. P. (1973). *Neuropharmac.*, **12**, 339–347.

WALTER, D. S. & ECCLESTON, D. (1972). *Biochem. J.*, **128**, 85–86P.

WALTER, D. S. & ECCLESTON, D. (1973). *J. Neurochem.*, **21**, 281–289.