studies. The contralateral hypotensive effect in the untreated fellow eye in our hypertensive subjects has been documented in animals (Green et al 1978) and suggests that the pressure lowering effect of Δ^9-THC is through systemic (cardiovascular and/or central nervous) rather than locally mediated effects within the eye. Before the full therapeutic potential of the cannabinoids are realized, further research should identify the ocular-active cannabinoid which would have minimal cardiovascular and psychologic effects so that local delivery systems of this cannabinoid may be devised.

REFERENCES

Crawford, W. J., Merritt, J. C. (1979) Int. J. Clin. Pharm. Biopharm. 17: 191–196

Crawford, W. J., Alexander, P. C., Merritt, J. C., Thombs, M. J., Curry, C. L. (1978) Prev. Med. 7: 54

Green, K., Bigger, J. F., Kim, K., Bowman, K. (1977) Exp. Eye Res. 24: 197–205

Green, K., Wynn, H., Bowman, K. A. (1978) Ibid. 27: 239–246

Merritt, J. C., Crawford, W. J., Alexander, P. C., Anduze, A. L., Gelbart, S. S. (1980) Ophthalmology 87: 222–228

J. Pharm. Pharmacol. 1981, 33: 41–43
Communicated July 11, 1980

0022–3573/81/010041–03 $0.250/0

Effect of GABAergic drugs on dopamine catabolism in the nigrostriatal and mesolimbic dopaminergic pathways of the rat

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Nigrostriatal and mesolimbic (mesocortical) dopaminergic neurons are involved in the regulation of muscle tone, extrapyramidal motility and general motor function, and are often affected in abnormal movement disorders (Hornykiewicz 1975) and psychotic disturbances (Stevens 1979). γ-Aminobutyric acid-utilizing (GABAergic) neurons are thought to interact with these dopaminergic systems and to regulate their activity (Fuxe et al 1975; Moore & Wuerthele 1979). There is good evidence for GABAergic neurons in both the nerve terminal and somatodendritic regions of the nigrostriatal dopaminergic pathway, viz. corpus striatum (Obata & Yoshida 1973; Bernardi et al 1976; Bartholini & Stadler 1977) and substantia nigra respectively (Precht & Yoshida 1971; Fonnum et al 1974; Hattori et al 1975; Ribak et al 1976). The role of GABAergic neurons in relation to the mesolimbic dopaminergic pathway is not as well elucidated, but the amino acid may similarly regulate the activity of these dopaminergic neurons both in the nucleus accumbens (Woodruff et al 1976; Pycock et al 1978; Beart et al 1980) and in the ventral tegmental area (Fonnum et al 1977; Wolf et al 1978; Walaas & Fonnum 1980). In Huntington's chorea a deficit of GABAergic neurons results in dopaminergic overactivity and consequent choreiform movements (Bird & Iversen 1974). The general involvement of GABA as an inhibitory neurotransmitter implies that GABAergic drugs could be of clinical importance in disorders such as Huntington's chorea, neuroleptic-induced tardive dyskinesias and epilepsy (Bartholini 1980; Meldrum 1978).

To analyse brain GABA-dopamine interactions we have examined the influence of several GABAergic drugs on the nigrostriatal and mesolimbic dopaminergic systems by studying drug-induced changes in the concentrations of the important dopamine metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC) (Roffler-Tarlov et al 1971). Alterations in the concentration of DOPAC represent a biochemical index of dopaminergic nerve activity and increases in DOPAC concentration are seen with increases in dopaminergic nerve impulse flow and vice-versa (Roth et al 1976).

Male Sprague-Dawley rats (150–250 g) were injected with drugs either intraperitoneally (2 ml kg^{-1}) or intravenously (1 ml kg^{-1}) into the tail vein. The following drugs were studied, picrotoxin (1 & 10 mg kg^{-1}, i.p., 60 and ca 15 min respectively), bicuculline (2 & 18 mg kg^{-1}, i.p., 60 min and ca 40 s respectively), 3-mercaptopropionic acid (15 & 90 mg kg^{-1}, i.p., 30, and ca 8 min respectively), aminooxyacetic acid hemi-hydrochloride (36 mg kg^{-1} of base, i.p., 60 min), 4-aminohex-5-ynoic acid (γ-acetylenic GABA 75 mg kg^{-1}, i.p., 240 min), nipecotic acid hydroxymethylpivalate oxalate (50 mg kg^{-1} of base, i.p., 60 min) and guvacine hydrobromide (40 mg kg^{-1} of base, i.v., 60 min). When convulsant doses were employed (picrotoxin, bicuculline and 3-mercaptopropionic acid) rats were killed at the onset of convulsions. Drugs were dissolved in 0·9% NaCl (saline) except for 4-aminohex-5-ynoic acid (distilled water) and bicuculline (1·1 equivalents of hydrochloric acid), and adjusted to pH 7·4 where appropriate. Corpus striatum, substantia nigra, nucleus accumbens and the ventral tegmental area were dissected as previously described (Beart & Gundlach 1980). Dissected brain areas were homogenized in 5 or 10 volumes of ice-cold 0·1 M hydrochloric acid, 0·1% EDTA and stored overnight at − 20 °C. Homogenates were centrifuged at 10 000 *g* for 5 min at 4 °C and portions of supernatants were assayed for DOPAC by a radioenzymatic assay employing catechol-*O*-methyltransferase and [^{3}H]-S-adenosyl methionine (Amersham) in which DOPAC is

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estimated after conversion to [^{3}H]homovanillic acid (Beart & Gundlach 1980).

Picrotoxin, a GABA antagonist, at a sub-convulsant dose (1 mg kg^{-1}, i.p., 60 min) produced a significant decrease in the concentration of DOPAC in corpus striatum, nucleus accumbens and the ventral tegmental area (Fig. 1). Conversely, a convulsant dose (10 mg kg^{-1}, i.p., ca 15 min) produced a significant elevation in the concentration of DOPAC in the same three regions (Fig. 1). By contrast, a convulsant dose of bicuculline (18 mg kg^{-1} i.p.), another GABA antagonist, failed to alter the concentration of DOPAC in the brain regions studied (data not shown). However, this lack of effect may be due to, the short time (40 s) before the onset of convulsions which might not allow for any functional change in dopamine turnover. A sub-convulsant dose of bicuculline (2 mg kg^{-1}, i.p., 60 min) also did not significantly change the concentration of DOPAC in all four brain regions (data not shown). Although convulsions themselves might alter DOPAC levels, experiments with a convulsant dose of leptazol (pentylenetetrazol 50 mg kg^{-1} i.p., 5 min), a drug which is not a GABA antagonist (Hill et al 1973), showed no effect on the concentrations of DOPAC in the corpus striatum and the ventral tegmental area (data not shown). Paradoxical effects have been noted with bicuculline and picrotoxin and it has been suggested that these may be due to distinct actions at different populations of GABA receptors (Leviel et al 1979).

GABA transaminase inhibitors reduced DOPAC concentrations in all brain regions (Fig. 1). The administration of amino-oxyacetic acid significantly lowered the concentration of DOPAC in corpus striatum with a substantial, but insignificant, decrease seen in the ventral tegmentum. At this dose aminooxyacetic acid is known to produce a large increase in the concentration of GABA with a concomitant enhancement of synaptically evoked inhibition (Gottesfeld et al 1972). Administration of 4-aminohex-5-ynoic acid, an effective in vivo inhibitor of GABA transaminase (Jung et al 1977), resulted in a significant reduction in DOPAC concentration in corpus striatum and ventral tegmentum. At the dose tested, both GABA transaminase inhibitors produced flaccidity and reduced motility. The results are in agreement with the observation that ethanolamine-*O*-sulphate, another GABA transaminase inhibitor, lowers brain dopamine turnover (Pycock et al 1978). 3-Mercaptopropionic acid, a potent inhibitor of the GABA synthesizing enzyme, glutamate decarboxylase, was studied at both subconvulsant and convulsant doses. Although a sub-convulsant dose of 3-mercaptopropionic acid (15 mg kg^{-1} i.p., 30 min) did not affect the concentration of DOPAC (not shown), a convulsant dose (90 mg kg^{-1} i.p., ca 8 min) significantly elevated DOPAC concentrations in both nerve terminal and somatodendritic regions of the mesolimbic pathway and produced similar, but non-significant, effects on the nigrostriatal system (Fig. 1). It has been reported by Swift et al (1978) that the latter dose of 3-mercaptopropionic acid effectively reduces GABA stores in substantia nigra which are associated with the GABAergic striatonigral pathway.

In a further series of experiments we examined the actions of GABA uptake inhibitors. Nipecotic acid (50 mg kg^{-1} i.p., 60 min) and guvacine (40 mg kg^{-1}, i.v., 60 min), two competitive inhibitors of the GABA uptake system (Johnston et al 1975), failed to alter the concentration of DOPAC in the four regions studied. Although GABA uptake inhibitors can enhance the effectiveness of electrophoretically administered GABA as an inhibitor of neuronal firing in vivo, they are unable to facilitate GABA-mediated synaptic inhibition (Lodge et al 1977). Such agents thus appear to be ineffective at functionally modulating GABA neurotransmission and hence altering dopamine turnover.

Overall the results of the present study reveal that drug treatments which block the action of synaptically released GABA (picrotoxin) or reduce control GABA concentrations (3-mercaptopropionic acid) can result in an elevation of DOPAC concentrations in the nerve terminal and/or somatodendritic regions of both dopaminergic pathways. Such an alteration in the levels of this dopamine metabolite has been shown to reflect increased activity in nigrostriatal and mesolimbic dopaminergic neurons (Roth et al 1976). Conversely drugs which increase GABA concentrations (amino-oxyacetic acid, 4-aminohex-5-ynoic acid) cause a lowering of the concentration of DOPAC due to decreased dopaminergic activity probably via increased synaptic inhibition (cf. Gottesfeld et al 1972). Thus

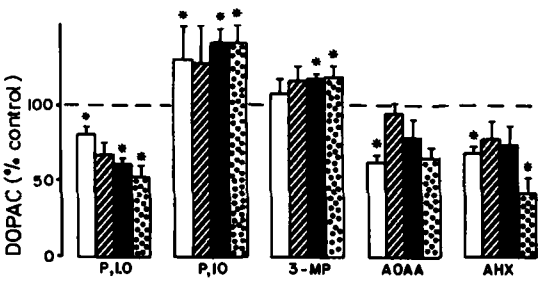

FIG. 1. Effect of picrotoxin (P, 1·0 and 10 mg kg^{-1}, i.p., 60 and 15 min respectively); 3-mercaptopropionic acid (3-MP, 90 mg kg^{-1} i.p., 8 min); amino-oxyacetic acid (AOAA, 36 mg kg^{-1} i.p., 60 min) and 4-aminohex-5-ynoic acid (AHX, 75 mg kg^{-1} i.p., 240 min) on the concentration of DOPAC in corpus striatum (open columns), substantia nigra (hatched columns), nucleus accumbens (solid columns) and ventral tegmentum (stippled columns). The results are expressed as percentage of control and are the mean ± s.e.m. of 4 animals. Control values are the mean ± s.e.m. of the number of animals indicated and were 1554 ± 104 (19); 297 ± 18 (21); 2185 ± 121 (21) and 427 ± 19 (22) ng DOPAC g^{-1} wet wt for corpus striatum, substantia nigra, nucleus accumbens and ventral tegmentum respectively. *$P < 0.05$ when compared with control (Student's *t*-test).

GABAergic neurons are likely to directly or indirectly inhibit the firing of both populations of dopaminergic neurons. In this context our observations with a low dose of picrotoxin would appear to be anomalous, but dose-dependent actions of picrotoxin have been previously reported (Worms et al 1978).

Our results indicate that certain GABAergic drugs can alter dopamine turnover and provide further evidence that GABA can modulate dopaminergic nerve activity in both the nerve terminal and somatodendritic regions of the nigrostriatal and mesolimbic pathways. The data indicate that GABA uptake inhibitors appear to be ineffective in this direction. However, our findings further strengthen the case for the development and clinical use of drugs which inhibit GABA transaminase in a specific and non-toxic fashion. Such GABA transminase inhibitors are likely to be of therapeutic benefit in treating disorders which involve dopaminergic overactivity, either alone or when co-administered with drugs such as neuroleptics.

This work was supported by a grant from the National Health and Medical Research Council of Australia. The authors are grateful to Dr G. A. R. Johnston (Sydney) and Dr P. Krogsgaard-Larsen (Copenhagen) for gifts of drugs.

REFERENCES

Bartholini, G. (1980) Trends in Pharmacological Sciences 1: 138–140

Bartholini, G., Stadler, H. (1977) Neuropharmacology 16: 343–347

Beart, P. M., Gundlach, A. L. (1980) Br. J. Pharmacol. 69: 241–247

Beart, P. M., Kuppers, D., Louis, W. J. (1980) Br. J. Pharmacol. 68: 160P–161P

Bernardi, G., Marciani, M. B., Morocutti, G., Giacomini, P. (1976) Brain Res. 102: 379–384

Bird, E. D., Iversen, L. L. (1974) Brain 97: 457–472

Fonnum, F., Grofova, I., Rinvik, E., Storm-Mathisen, J., Walberg, F. (1974) Brain Res. 71: 77–92

Fonnum, F., Walaas, I., Iversen, E. 1(977) J. Neurochem. 29: 221–230

Fuxe, K., Hökfelt, T., Ljungdahl, A., Agnati, L., Johansson, O., Perez de la Mora, M. (1975) Med. Biol. 53: 177–183

Gottesfeld, Z., Kelly, J. S., Renaud, L. P. (1972) Brain Res. 42: 319–335

Hattori, T., Fibiger, H. C., McGeer, P. L. (1975) J. Comp. Neurol. 162: 487–504

Hill, R. G., Simmonds, M. A., Straughan, D. W. (1973) Br. J. Pharmacol. 49: 37–51

Hornykiewicz, O. (1975) Biochem. Pharmacol. 24: 1061–1065

Jung, M. J., Lippert, B., Metcalf, B. W., Schecter, P. J., Böhlen, P., Sjoerdsma, A. (1977) J. Neurochem. 28: 717–723

Johnston, G. A. R., Krogsgaard-Larsen, P., Stephanson, A. (1975) Nature (London) 258: 627–628

Leviel, V., Cheramy, A., Nieoullon, A., Glowinski, J. (1979) Brain Res. 175: 259–270

Lodge, D., Johnston, G. A. R., Curtis, D. R., Brand, S. J. (1977) Brain Res. 136: 513–522

Meldrum, B. S. (1978) Lancet 2: 304–306

Moore, K. E., Wuerthele, S. M. (1979) Progr. Neurobiol. 13: 325–359

Obata, K., Yoshida, M. (1973) Brain Res. 64: 455–459

Precht, W., Yoshida, M. (1971) Ibid. 32: 229–233

Pycock, C. J., Horton, R. W., Carter, C. J. (1978) in: Roberts, P. J., Woodruff, G. N., Iversen, L. L. (eds) Adv. Biochem. Psychopharmacol. Raven Press, New York, 19: 323–341

Ribak, C. E., Vaughn, J. E., Saito, K., Barber, R., Roberts, E. (1976) Brain Res. 116: 287–298

Roffler-Tarlov, S., Sharman, D. F., Tergerdine, P. (1971) Br. J. Pharmacol. 42: 343–351

Roth, R. H., Murrin, C., Walters, J. R. (1976) Eur. J. Pharmacol. 36: 163–171

Stevens, J. (1979) Trends in Neuroscience 2: 102–105

Swift, R. M., Hoffmann, P. C., Heller, A. (1978) Brain Res. 156: 181–186

Walaas, I., Fonnum, F. (1980) Neuroscience 5: 63–72

Wolf, P., Olpe, H. R., Avrith, D., Haas, H. L. (1978) Experientia 34: 73–74

Woodruff, G. N., McCarthy, P. S., Walker, R. J. (1976) Brain Res. 115: 233–242

Worms, P., Willigens, M. T., Lloyd, K. G. (1978) J. Pharm. Pharmacol. 30: 716–718