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Increased brain concentration of homovanillic acid in rats treated with *threo*-3,4-dihydroxyphenylserine

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Threo-3,4-Dihydroxyphenylserine (DOPS) is a synthetic amino acid precursor of noradrenaline (NA) (Creveling et al 1968). Although it is a rather poor substrate for the decarboxylase that converts it to NA (Goodwin et al 1972), it nevertheless has been a useful pharmacological tool for selectively elevating brain NA concentrations. Recently, we observed that while DOPS causes an increase in the brain concentration of 3-methoxy-4-hydroxyphenethylene glycol (MHPG), a neutral metabolite of NA, it unexpectedly causes an even greater percent elevation in the brain concentrations of the neutral dopamine (DA) metabolites, 3-methoxy-4-hydroxyphenethanol (MHPE) and 3,4-dihydroxyphenethanol (DHPE) (Edwards & Rizk 1981a). Since the neutral metabolites of DA, in contrast to those of NA, represent only minor metabolites (Edwards et al 1981; Edwards & Rizk 1981b), it was of interest to determine whether DOPS similarly increases the concentrations of the acidic DA metabolites.

An experiment was done in which brain levels of the acidic DA metabolite, homovanillic acid (HVA), were measured in control and DOPS-treated rats, which had been pretreated with 0·9% NaCl (saline) or Ro 4-4602 [N^1-(DL-seryl)-N^2-(2,3-trihydroxybenzyl)hydrazine], a peripheral decarboxylase inhibitor.

Six-week old male Sprague-Dawley rats (Zivic-Miller Labs, Allison Park, PA), 200–250 g, were divided into four groups matched by weight. They were injected i.p. with 50 mg kg^{-1} Ro 4-4602 or saline between 10.00 and 11.00 a.m. and 1 h later with either 0·05 M HCl or 250 mg kg^{-1} DOPS (± threo-DOPS, Sigma Chemical Co., St Louis, MO). The animals were decapitated 1 h after the second injection and the brains rapidly removed and frozen on dry ice. Free HVA was assayed by gas chromatography-mass spectrometry in the chemical ionization mode (Edwards 1982).

The data in Table 1 show that a dose of 250 mg kg^{-1} of DOPS caused an 81% increase in the brain concentration of HVA ($P < 0·001$). However, only a 19% increase ($P < 0·05$) was observed in Ro 4-4602 pretreated animals, suggesting that most of the increase in HVA induced by DOPS occurred in brain capillaries or possibly at sites outside the brain. Unexpectedly, Ro 4-4602 pretreatment alone caused a significant increase in brain HVA levels of 33% ($P < 0·05$). The cause of this effect is unknown.

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Table 1. Whole brain HVA concentrations in DOPS-treated rats. ± threo-DOPS (250 mg kg^{-1}) and Ro 4-4602 (50 mg kg^{-1}) were injected i.p. one and two hours, respectively, before the animals were killed. All values represent the mean ± s.e.m. for the number of animals indicated in parentheses. Statistical analyses were performed using Student's *t*-test.

Treatment	Brain HVA concentration (ng g^{-1})
Control	73·5 ± 5·2 (5)
DOPS	133·1 ± 5·0** (5)
Ro 4-4602	97·9 ± 6·3* (5)
Ro 4-4602 + DOPS	116·9 ± 0·9‡ (4)

* $P < 0·05$ vs control; ** $P < 0·001$ vs control.
‡ $P < 0·05$ vs Ro 4-4602 alone.

These results are consistent with our previous observation that DOPS raises the brain levels of the minor DA metabolites, DHPE and MHPE (Edwards & Rizk 1981a). Since HVA, a major DA metabolite, is also increased, this rules out the possibility that the effect is specific to the metabolism of the neutral metabolites. Instead, the present data suggest that DOPS has a more general effect on DA systems.

One apparent difference between the effects of DOPS on HVA and on the neutral metabolites is that the elevation in HVA was partially blocked by pretreatment with Ro 4-4602 (Table 1), whereas the DOPS-induced increases in the levels of the neutral metabolites were either unaffected or even enhanced by pretreatment with this peripheral decarboxylase inhibitor (Edwards & Rizk 1981a). This supports the idea that the neutral DA metabolites may be preferentially formed within the central nervous system and therefore may represent useful indicators of central DA function (Edwards et al 1981).

The most likely explanation for the increases in brain concentrations of DA metabolites is that DOPS is decarboxylated to NA in DA neurons, thereby causing a release of DA and subsequently an elevation in the levels of its metabolites. Apparently, this effect of NA is not limited to the naturally occurring isomer, since (+)-*erythro*-DOPS, which is decarboxylated to yield the unnatural (+) form of NA, has also been reported to cause an increase in the concentration of HVA in the rat brain (Bartholini et al 1975).

Our findings are analogous to those of Fuller & Perry (1981), who observed that the brain levels of 3,4-

dihydroxyphenylacetic acid (DOPAC), another DA metabolite, were increased up to sixfold by 5-hydroxytryptophan, the amino acid precursor of 5-hydroxytryptamine (5-HT). They suggested that this effect might be due to the displacement of DA by 5-HT formed in dopaminergic neurons. Similarly, it is possible that increases in DA metabolites that have been reported following tyrosine injections (Sved et al 1979; Edwards 1982) result, in part, by the indirect actions of tyramine formed in DA neurons.

In summary, we have demonstrated that DOPS produces an increase in the brain levels of DA metabolites. Caution should be used before any behavioral or pharmacological effects of DOPS are attributed solely to the function of NA neurons.

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REFERENCES

Bartholini, G., Constantinidis, J., Puig, M., Tissot, R., Pletscher, A. (1975) J. Pharmacol. Exp. Ther. 193: 523–532

Creveling, C. R., Daly, J., Tokuyama, T., Witkop, B. (1968) Biochem. Pharmacol. 17: 65–70

Edwards, D. J. (1982) Life Sci. 30: 1427–1434

Edwards, D. J., Rizk, M. (1981a) Prog. Neuro-Psychopharmacol. 5: 569–572

Edwards, D. J., Rizk, M. (1981b) J. Neurochem. 36: 1641–1647

Edwards, D. J., Rizk, M., Spiker, D. G. (1981) Biochem. Med. 25: 135–148

Fuller, R. W., Perry, K. W. (1981) J. Pharm. Pharmacol. 33: 406–407

Goodwin, B. L., Johnson, R. D., Leask, G. S., Ruthven, C. R. J., Sandler, M. (1972) Experientia 28: 1298–1299

Sved, A. F., Fernstrom, J. D., Wurtman, R. J. (1979) Life Sci. 25: 1293–1300

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LETTER TO THE EDITOR

In vitro and in vivo effects of pentobarbitone sodium

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There is now good evidence that the anaesthetic properties of barbiturates may be due, in part, to an enhancement of synaptic inhibitions in the central nervous system mediated by γ-aminobutyric acid (GABA) (Nicoll 1980). Recently pentobarbitone sodium has been demonstrated to enhance the binding of [^{3}H]GABA to rat brain synaptosomal membranes (Willow & Johnston 1980), in agreement with the observed effects of this agent on the inhibition of the firing rate of feline dorsal horn interneurons by electrophoretically administered GABA (Lodge & Curtis 1978). We report here the comparative pharmacological effects of two batches of pentobarbitone sodium (PB-A and PB-B respectively) obtained from two commercial sources.

PB-A produced light and inconsistent anaesthesia in cats following intraperitoneal (i.p.) administration of 35 mg kg^{-1}, and the dose required to produce loss of the righting reflex in 50% male mice (ED50) was 43 ± 1 mg kg^{-1} (i.p.) (calculated by probit analysis, n = 20 per dose group). The induction of anaesthesia in mice was preceded by hyperexcitability, characterized by front and hind limb extension and arching of the back. At a concentration of 100 μM, PB-A did not enhance GABA binding to rat brain synaptosomal membranes, under the conditions used by Willow & Johnston (1980).

On the other hand, PB-B was an effective anaesthetic of cats at a dose of 35 mg kg^{-1} (i.p.). The ED50 in mice for this compound was 33 ± 1 mg kg^{-1} (i.p.), and there were no signs of pre-anaesthetic excitation. At 100 μM, PB-B enhanced GABA binding to rat brain synaptosomal membranes by 35 ± 2%. Furthermore, PB-B, when administered electrophoretically or systemically, was effective in enhancing the effects of electrophoretic GABA on feline dorsal horn interneurons (Lodge & Curtis 1978).

Chemical analyses using high pressure liquid chromatography, infrared spectroscopy, optical rotation, and thin layer chromatography suggested that PB-A and PB-B were identical, and both samples conformed with internationally accepted standards for pentobarbitone as a chemical. This suggests that if the anomalous effects of PB-A were due to a trace contaminant, then such a substance was highly potent in antagonizing the anaesthetic actions of pentobarbitone. While at present there is no conclusive explanation for differences between the actions of PB-A and PB-B, it seems essential that the source of samples of pentobarbitone be identified in all publications concerned with its biological or neurochemical effects. Furthermore, reliance on chemical analysis alone may be inadequate for the biological specification of this barbiturate.

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REFERENCES

Lodge, D., Curtis, D. R. (1978) Neurosci. Lett. 8: 125–129

Nicoll, R. A. (1980) in: Iversen, L. L., Iversen, S. D., Snyder, S. H. (eds) Handbook of Psychopharmacology, Vol. 12, Plenum Press: New York, pp 187–234

Willow, M., Johnston, G. A. R. (1980) Neurosci. Lett. 18: 323–327