p-HYDROXYNOREPHEDRINE: ITS SELECTIVE DISTRIBUTION IN DIFFERENT RAT BRAIN AREAS

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THE MECHANISMS proposed for the explanation of the various effects elicited by *d*-Amphetamine (*d*-A) invoke either the direct action of *d*-A on norepinephrine (NE) or dopamine (DA) stores, or the accumulation of one of its metabolites: *p*-Hydroxynorephedrine (*p*OHNE) (BRODIE *et al.*, 1970; COSTA and GROPPETTI, 1970; SULSER and SANDERS-BUSH, 1971; BOAKES, 1972). In fact the depletion of heart and brain NE outlasts for more than 24 hr the *d*-A disappearance from these tissues, whereas *p*OHNE accumulates in heart and brain of rats and its concentrations have a half life of about 20 hr (COSTA and GROPPETTI, 1970).

The elucidation of the central role of *p*OHNE seems therefore to be of importance, because if more is known about its formation, localization and disappearance from different brain areas, more light would be shed on the mechanisms underlying effects such as a tachyphylaxis and psychosis in man after *d*-A treatment.

It has been suggested, on the basis of results obtained in peripheral nervous system, that also in brain *p*OHNE may be synthetised from *p*-Hydroxyamphetamine (*p*OHA) (GOLDSTEIN and ANAGNOSTE, 1965) and selectively stored in noradrenergic nerve endings, acting as a false neurotransmitter (FISHER *et al.*, 1965; THOENEN *et al.*, 1966; GROPPETTI and COSTA, 1968).

Since only circumstantial evidence has been offered in support of this hypothesis, mainly because the analytical methods available did not have sufficient specificity and sensitivity, we have developed a mass fragmentographic assay for *p*OHNE at picomole levels (CATTABENI *et al.* (1973) in press).

Utilising this technique, we have measured *p*OHNE levels after 6-hydroxydopamine (6-OHDA) treatment. 6-OHDA has shown to be a useful tool in the study of the noradrenergic system function. Evidences from morphological studies suggest that this compound causes an ultra-structural modification of the axons and nerve terminals of the sympathetic nervous system. When injected intracisternally or into the lateral ventricles of rat brain, 6-OHDA produces a selective and long lasting depletion of brain NE while dopaminergic and serotonergic nerve terminals are only slightly affected (TRANZER and THOENEN, 1968; UNGERSTEDT, 1968; URETSKY and IVERSEN, 1969; BLOOM *et al.*, 1969). In our experiment, the animals were given intraventricularly 200 μg per rat of 6-OHDA and after one week *d*-A (7 mg/kg i.p.) and sacrificed 5 hr later.

In treated animals *p*OHNE levels were 60 per cent lower when compared with controls and, since brain NE concentrations were also decreased by a similar extent, while 80 per cent of DA was still present, it seems appropriate to infer that also in brain *p*OHNE is associated with the noradrenergic system. To further substantiate

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the relationship between the noradrenergic system and *p*OHNE formation and/or storage, we have studied the distribution of this compound in different brain areas after high doses of *d*-A.

As expected (see Table 1) only traces of *p*OHNE are present in the caudate nucleus, whereas consistent levels of *p*OHNE are observed in the hypothalamus and

TABLE 1. CORRELATION BETWEEN THE DISTRIBUTION OF *p*-OH-NOREPHEDRINE (*p*OHNE) IN BRAIN AREAS OF AMPHETAMINE TREATED RATS* AND NOREPINEPHRINE (NE) LEVELS IN CONTROL ANIMALS

Brain area	*p*OHNE μg/g $\pm$ S.E.	NE† μg/g $\pm$ S.E.	*p*OHNE/NE
Cerebellum	0·192 $\pm$ 0·017	0·18 $\pm$ 0·01	1:1
Tel-diencephalon	0·175 $\pm$ 0·044	0·46 $\pm$ 0·01	1:2·5
Brain stem	0·172 $\pm$ 0·029	0·71 $\pm$ 0·03	1:4
Hypothalamus	0·420 $\pm$ 0·072	2·10 $\pm$ 0·13	1:5
Caudate N.	0·040 $\pm$ 0·004	n.d.	

* *d*-Amphetamine 7 mg/kg i.p., 5 hr before sacrifice.

† NE was measured spectrophotofluorimetrically according to C. C. CHANG, *J. Neuropharm.* 3, 643 (1964).

n.d.: not detectable.

The values are a mean $\pm$ S.E. of 3 experiments.

other brain areas rich in NE. Considering the *p*OHNE to NE ratio, as indicated in the Table, it is possible to infer that *p*OHNE synthesis and/or storage occur preferentially at nerve endings rather than in cell bodies. In fact the ratio is higher in brain structures containing predominantly nerve terminals, as in the cerebellum and tel-diencephalon. Obviously, it would be more appropriate to relate *p*OHNE levels to the functional activity of noradrenergic nerve endings in different brain areas rather than to NE levels. Unfortunately indications on turnover rate in all these brain areas are still incomplete.

We are now investigating whether this preferential accumulation of *p*OHNE is time dependent and if the rate of disappearance follows the same time course in different brain areas, in the hope to shed more light on the possible role played by *p*OHNE in the behavioural effects that follow *d*-A treatment.

Preliminary data have been obtained also for the levels of *p*OHA, the other hydroxylated metabolite of *d*-A in several species, including man. GOLDSTEIN and ANAGNOSTE (1965) have demonstrated that this compound can be in turn utilised as a substrate for dopamine-β-hydroxylase to form *p*OHNE.

We have observed that after *d*-A administration the highest concentrations of *p*OHA are present in caudate nucleus. In brain stem, cerebellum and tel-diencephalon the levels of this metabolite are only 50 per cent of those found in the caudate.

CARLSSON (1970) and COSTA *et al.*, (1972) have suggested that an action on dopaminergic neurones in caudate nucleus may be entertained as a possible indirect mechanism involved in hypermotility elicited by *d*-A in rats. Therefore it is tempting to speculate that *p*OHA could play a role in this behavioural effect.

In conclusion, the selective distribution of *p*OHNE in different rat brain areas indicates that also in the central nervous system this *d*-A metabolite is associated with noradrenergic nerve endings.

Preliminary results indicate that also *p*OHA has a preferential distribution in rat

brain areas, being localised maximally in the caudate nucleus. Experiments are in progress to ascertain if these compounds can be involved in the mechanism of some of the behavioural and biochemical effects due to *d*-A treatment.

REFERENCES

BLOOM F. E., ALGERI S., GROPPETTI A., REVUELTA A. and COSTA E. (1969) *Science* **166,** 1284–1286.
BOAKES R. J., BRADLEY P. B. and CANDY J. M. (1972) *Br. J. Pharmacol.* **45,** 391–403.
BRODIE B. B., CHO A. K. and GESSA G. L. (1970) In: *International Symposium on Amphetamines and Related Compounds* (E. COSTA and S. GARATTINI, Eds.), pp. 217–230, Raven Press, New York.
CARLSSON A. (1970) In: *International Symposium on Amphetamines and Related Compounds* (E. COSTA and S. GARATTINI, Eds.), pp. 289–300, Raven Press, New York.
CATTABENI F., RACAGNI G. and GROPPETTI A. (1973). (in press) In: *Advances in Biochemical Psychopharmacology* Vol. 7 (E. COSTA and B. HOLMSTEDT, Eds), Raven Press, New York.
COSTA E. and GROPPETTI A. (1970) In: *International Symposium on Amphetamines and Related Compounds* (E. COSTA and S. GARATTINI, Eds), pp. 231–255, Raven Press, New York.
COSTA E., GROPPETTI A. and NAIMZADA K. (1972) *Br. J. Pharmacol.* **44,** 742–751.
FISHER J. E., HORST W. D. and KOPIN I. J. (1965) *Br. J. Pharmacol.* **24,** 477–484.
GOLDSTEIN M. and ANAGNOSTE B. (1965) *Biochim. Biophys. Acta* **107,** 166–168.
GROPPETTI A. and COSTA E. (1968) *Atti Accademia Medica Lombarda* **23,** 1105–1107.
SULSER F. and SANDERS-BUSH E. (1971) *Ann. Rev. Pharmacol.* **11,** 209–230.
THOENEN H., HURLIMAN K. F., GEY K. F. and HAEFELY W. (1966) *Life Sci.* **5,** 1715–1722.
TRANZER J. P. and THOENEN H. (1968) *Experientia* **24,** 155–156.
UNGERSTEDT U. (1968) *Europ. J. Pharmacol.* **5,** 107–110.
URETSKY N. J. and IVERSEN L. L. (1969) *Nature, Lond.* **221,** 557–559.