MARIN, W. R. (1970). *Psychopharmacologia (Berl.)*, **17**, 242–257.
MONACHON, M.-A., BURKARD, W. P., JALFRE, M. & HAEFELY, W. (1972). *Arch. Pharmac.*, **274**, 192–197.
NEREBSKI, J., ROMANOWSKI, W. & KADJIELA, W. (1962). *Acta physiol. pol.*, **14**, 157–170.
NYBACK, H. & SEDVALL, G. (1968). *J. Pharmac. exp. Ther.*, **162**, 294–301.

Interaction of β-phenethylamine with dopamine and noradrenaline in the central nervous system of the rat

G. B. BAKER†, M. RAITERI*, A. BERTOLLINI*, R. DEL CARMINE*, P. E. KEANE, I. L. MARTIN, *MRC Neuropharmacology Unit, The Medical School, Birmingham B15 2TJ, U.K. and *Istituto di Farmacologia, Universita Cattolica, Via Pineta Sacchetti 644, Rome, Italy*

Pretreatment of rodents with β-phenethylamine (PE) has been reported to result in decreased brain catecholamine concentrations (Jonsson, Grobecker & Holtz, 1966; Fuxe, Grobecker & Jonsson, 1967; Jackson & Smythe, 1973). Jonsson & others (1966) further stated that the observed decrease in noradrenaline was largely confined to the particulate fraction of rat brain. However, the mechanism by which PE interacts with dopamine and noradrenaline in the central nervous system remains unclear. We report the results of a series of experiments *in vivo* and *in vitro* designed to investigate the interaction of PE with transport of catecholamines in brain regions rich in dopamine or noradrenaline.

Experiments *in vivo* were carried out on male Wistar rats (250–300 g) anaesthetized with urethane (1·8 g kg^{-1}). Rats were prepared for intracaudate or intrathalamic perfusion by the placement of a push-pull cannula in the caudate-putamen or thalamus respectively (coordinates: caudate-putamen, A = 7·8, L = 3·0, H = 1·0, thalamus, A = 3·0, L = 2·8, H = 0·0 according to the atlas of de Groot). [Ethylamine-1,2-^{3}H]dopamine (^{3}H-DA, 5 μCi Amersham, sp. act. 3·9 Ci mmol^{-1}) or 1- [7-^{3}H]noradrenaline (^{3}H-NA, 5 μCi, Amersham, 10·3 Ci mmol^{-1}) as the hydrochloride and acetate respectively were injected in a volume of 1·0 μl of artificial cerebrospinal fluid (csf) (Besson, Cheramy & others, 1971) at a point 1 mm below the cannula tip. After 30 min, the region was perfused with artificial csf containing nialamide (1×10^{-6} M), at a rate of 3 ml h^{-1}. The perfusion was continued for 2 h, after which the perfusate was collected at 2 min intervals for 20 min. At this time, PE (1×10^{-5} M) as hydrochloride was added to the perfusing medium for 2 min, and the collection of 2 min samples continued for a further 20 min. The amount of radioactivity in each sample was measured by liquid scintillation counting. When the brain regions were perfused there was a 3–4 fold increase above baseline concentrations in the efflux of dopamine from the caudate in each of the two fractions collected following perfusion of PE and a 2–3 fold increase of noradrenaline in these same two fractions collected from the thalamus, followed by a return to baseline concentrations. Experiments with [^{14}C]urea indicated that there was also substantial non-specific efflux after perfusion of PE.

In some of the *in vivo* experiments, a ventricular perfusion method was used. An 'inflow' cannula was placed in the lateral ventricle and an 'outflow' cannula in the third ventricle. 60 min after injection of ^{3}H-DA or ^{3}H-NA (5 μCi) into the inflow cannula, perfusion with artificial csf was carried out for 1·5 h at a rate of 4·5 ml h^{-1}. This was followed by collection of the perfusates in 2 min intervals as described above. In this ventricular perfusion study [^{14}C]urea efflux was virtually abolished while there was still a dose-dependent efflux of ^{3}H-DA and ^{3}H-NA following perfusion of PE (a 2-fold increase of ^{3}H-DA and ^{3}H-NA efflux over baseline values after addition of 10^{-5}M PE).

The urea efflux observed is in agreement with the results of other authors. Using the push-pull cannulation technique, Chase & Kopin (1968) also found efflux of [^{14}C]urea caused by stimuli which increased [^{3}H]catecholamine release, while Von Voigtlander & Moore (1973) reported that this release of metabolically inert substances was greatly reduced by using a ventricular perfusion method.

Experiments on uptake and release *in vitro* were conducted in synaptosome-enriched P_2 fractions (Gray & Whittaker, 1962) using methods previously described (Levi & Raiteri, 1973, Raiteri, Angelini & Levi, 1974a). Briefly, this consisted of incubating P_2 fractions with ^{3}H-DA or ^{3}H-NA (in a final concentration of 1×10^{-7}M) for 10 min at 37°. In uptake studies PE was added together with the radioactive substrate at the beginning of the incubation; aliquots of the incubation mixture were then centrifuged in a microfuge (Beckman, Model 152B), the pellets were washed rapidly at 37°, and the radioactivity of perchloric acid extracts counted. To study release, aliquots of the preloaded P_2 suspension were placed on Millipore filters, washed rapidly at 37°, and superfused with oxygenated glucose-containing medium with or without added PE. Nialamide ($1{\cdot}25 \times 10^{-5}$M) was present in the incubation and superfusion medium.

The results of the studies *in vitro* are shown in Fig. 1. While PE caused considerable increase in the release of ^{3}H-DA from striatal synaptosomes (133% increase during 5 min) (a), it was a much weaker stimulator (38% increase) of ^{3}H-NA release from hypothalamic

† Correspondence.

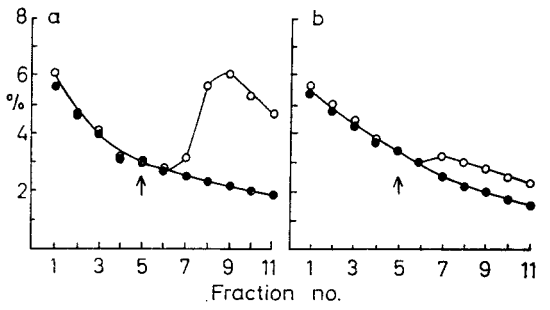

FIG. 1. Effect of β-phenethylamine (10^{-5}M) on release of [^{3}H]dopamine from P_2 fractions prepared from corpus striatum (a) and on release of [^{3}H]noradrenaline from P_2 fractions prepared from hypothalamus (b); ●, control values; ○, β-phenethylamine added during the superfusion; ↑, point at which β-phenethylamine was added. The y-axis represents the % of total radioactivity (fractions & filter) present in each fraction after the superfusion period. Each point on the graphs is the mean of three experiments.

synaptosomes (b). A comparison of uptake results revealed that while PE was only a moderate inhibitor of ^{3}H-DA uptake in corpus striatum (36% inhibition at 10^{-5}M, no inhibition at 10^{-6}M), it was a stronger inhibitor of ^{3}H-NA uptake in the hypothalamic preparations (75% inhibition at 10^{-5}M PE, 45% at 10^{-6}M and 14% at 10^{-7}M).

As Von Voigtlander & Moore (1973) have pointed out, in conventional experiments using perfusion, even though the addition of a drug to the perfusion medium may result in an increase in the efflux of neurotransmitter, this cannot correctly be interpreted as release since the effect observed may be due either to true release or to an inhibition of reuptake. The superfusion technique used in the work *in vitro* described here minimizes reuptake effects (Raiteri & others, 1974a; Raiteri, Levi & Federico, 1974b) and thus the peaks shown in Fig. 1(a) and (b) represent true release. On the basis of the results of these experiments, it appears that PE can interact with dopamine and noradrenaline, causing release and an inhibition of reuptake of both neurotransmitters. However, the releasing action is the predominant effect with dopamine, while inhibition of reuptake is the principal action on noradrenaline. Horn (1973) has previously reported that PE is a more effective inhibitor of dopamine uptake in homogenates of corpus striatum than of noradrenaline in hypothalamus, but his work was done in rats which had been treated with reserpine.

These effects of PE on dopamine and noradrenaline uptake and release may assume considerable importance following administration of drugs which increase brain PE concentrations. This interaction with catecholamine transport could also account for some of the observations on the stimulant effects of injected PE on locomotor activity. Jackson (1974) has stated that the increased locomotor activity observed in mice 5–15 min after injection of PE (50 mg kg, i.p.) is dependent upon an intact dopamine and noradrenaline synthetic pathway

The authors wish to thank Professor P. B. Bradley and Dr G. B. Ansell for their advice and encouragement in the work. We gratefully acknowledge the technical assistance of Mr F. Angelini, Miss L. Tovell and Mr S. Weston. G. B. B. wishes to thank the Canadian M.R.C. for continuing financial support.

November 4, 1975

REFERENCES

BESSON, M. J., CHERAMY, A., FELTZ, P. & GLOWINSKI, J. (1971). *Brain Res.*, **32**, 407–424.
CHASE, T. N. & KOPIN, I. J. (1968). *Nature*, **217**, 466–467.
DE GROOT, J. (1959). *The Rat Forebrain in Stereotoxic Coordinates*. Amsterdam: N. V. North Holland.
FUXE, K., GROBECKER, H. & JONSSON, J. J. (1967). *Eur. J. Pharmac.*, **2**, 202–207.
GRAY, E. G. & WHITTAKER, V. P. (1962). *J. Anat.*, **96**, 79–88.
HORN, A. S. (1973). *Br. J. Pharmac.*, **47**, 322–338.
JACKSON, D. M. (1974). *J. Pharm. Pharmac.*, **26**, 651–654.
JACKSON, D. M. & SMYTHE, D. B. (1973). *Neuropharmac.*, **12**, 663–668.
JONSSON, J., GROBECKER, H. & HOLTZ, P. (1966). *Life Sci.*, **5**, 2235–2246.
LEVI, G. & RAITERI, M. (1973). *Brain Res.*, **57**, 165–185.
RAITERI, M., ANGELINI, F. & LEVI, G. (1974a). *Eur. J. Pharmac.*, **25**, 411–414.
RAITERI, M., LEVI, G. & FEDERICO, R. (1974b). *Ibid.*, **28**, 237–240.
VON VOIGTLANDER, P. F. & MOORE, K. E. (1973). *J. Pharmac. exp. Ther.*, **184**, 542–552.