Pakkenberg & Randrup (1968) and tested by the method of Costall & Olley (1971), was not induced in these rats but prostration, an atonic depressed appearance with the animal still retaining its desire and capacity to move from an abnormal position, was detected and studied in animals after cannabis pretreatment. It was found that only the doses exceeding 1 ml kg^{-1} tincture of cannabis B.P.C. induced signs of prostration, the highest dose (10 ml kg^{-1}) grossly affecting all the animals.

Thus cannabis has been differentiated from neuroleptic cataleptogenic agents by its complex interaction with amphetamine.

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School of Pharmacology,
University of Bradford,
Bradford BD7 1DP,
West Yorkshire, U.K.

A. L. GOUGH
J. E. OLLEY

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REFERENCES

ARNFRED, T. & RANDRUP, A. (1968). *Acta pharmac. tox.*, **26**, 384–394.
COSTALL, B. & NAYLOR, R. J. (1973a). *Archs Pharmac.*, **278**, 117–133.
COSTALL, B. & NAYLOR, R. J. (1973b). *Eur. J. Pharmac.*, **24**, 8–24.
COSTALL, B. & OLLEY, J. E. (1971). *Neuropharmac.*, **10**, 297–306.
MUNKVAD, I., PAKKENBERG, H. & RANDRUP, A. (1968). *Brain Behav. Evol.*, **1**, 89–100.
NAYLOR, R. J. & OLLEY, J. E. (1972). *Neuropharmac.*, **11**, 91–99.
PERTWEE, R. H. (1972). *Br. J. Pharmac.*, **46**, 753–763.
RANDRUP, A. & MUNKVAD, J. (1967). *Psychopharmacologia (Berl.)*, **11**, 300–310.
TAYLOR, K. M. & SNYDER, S. H. (1971). *Brain Res.*, **28**, 295–309.
TAYLOR, W. A. & SULSER, F. (1973). *J. Pharmac. exp. Ther.*, **185**, 620–632.

Accumulation of acetylcholine and aromatic monoamines by interaction with adenosine-5′-triphosphate

Biogenic monoamines, such as, for example, catecholamines and 5-hydroxytryptamine, are thought to accumulate in subcellular storage organelles by two different mechanisms, i.e. by an active transport at the level of the granular membrane and by interaction with nucleotides present in high concentration in the organelles (intragranular mechanism) (Pletscher, Da Prada & others, 1974). Evidence of accumulation by interaction with adenosine-5′-triphosphate (ATP) has been presented in a diffusion system consisting of two chambers separated by an artificial lipid membrane impermeable to ATP. One of the chambers contained ATP, the other did not. In this system, noradrenaline originally present in equal concentration in both chambers accumulated against an apparent concentration gradient in the chamber containing ATP (Berneis, Da Prada & Pletscher, 1974).

In the present work, these experiments have been extended to other amines using a new system for equilibrium dialysis with an artificial non-lipid containing membrane. In this system, in contrast to that used before, the passage of the amines through the membrane was rapid and unhindered, and the diffusion of amines with low lipid solubility (e.g. acetylcholine) could also be determined.

A Dianorm equilibrium dialyser (Diachema AG, Birmensdorf/Zurich, Switzerland) made of Teflon and containing two microchambers separated by a "spectropor 3TM membrane" (M.W. cut-off: 3500) (Spectrum Medical Industries Inc., Los Angeles, USA) (Fig. 1) was used for all experiments. Both chambers were filled with the same

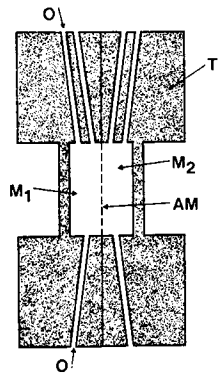

FIG. 1. Model of microdialyser. AM = artificial membrane. M_1 and M_2 = microchambers of 200 μl each. O = open outlet (plugged during incubation) for filling or removal of incubation mixture. T = Teflon. After filling, 5 dialysers were fixed on a rotor and submerged in a water bath in order to maintain a constant temperature (Da Prada & others, 1975).

aqueous solution of a radioactively labelled amine or amino acid, chamber M_2 also containing ATP (disodium salt; initial concentration 200 mM). The pH of both chambers was adjusted to 6·5. These operations were carried out at 2°.

Equilibrium dialysis experiments were performed at 37° and 2° at various incubation times and amine concentrations. At the end of each experiment, 40 μl aliquots in duplicate were removed from each chamber, placed into 10 ml of a scintillation cocktail (Aquasol, New England Nuclear, Boston, USA) and counted in an Isocap/300 liquid scintillation system (Nuclear Chicago, USA). The quotient: amine concentration in chamber M_2 divided by that in chamber M_1 is taken as the concentration gradient of the amine.

Figure 2 shows that on incubation of dopamine, histamine and acetylcholine (initial molar concentration ratio amine to ATP = 0·1) a concentration gradient between chamber M_2 (originally containing 200 mM ATP) and chamber M_1 (originally devoid of the nucleotide) built up. At 37°, the gradient reached a maximum and then decreased. The maximum was highest for histamine, followed in decreasing order by dopamine and acetylcholine. When the initial concentration of ATP in chamber M_2 was kept constant (200 mM), variations of the initial molar ratio dopamine to ATP from 2·5 to 0·1 caused a gradual increase of the maximum amine concentration gradient from 1·3 to 2·8. At 2° the establishment of the concentration gradient was slower than at 37°, maximum values being attained after about 30 min. All three amines showed higher maxima at 2° than that at 37°, while the order of the maxima remained the same (Fig. 2). Other amines incubated similarly also built up a concentration gradient. The maximum values (averages with s.e.m., n = 3–4), which at 37° were attained after 10 min, amounted to 2·07 $\pm$ 0·03 for (−)-noradrenaline (methylene[^{14}C]D-bitartrate), 2·25 $\pm$ 0·12 for 5-hydroxytryptamine (side chain -1-^{14}C, creatinine sulphate), 3·01 $\pm$ 0·19 for tyramine (side chain -1-^{14}C, HCl), 3·12 $\pm$ 0·19 for tryptamine (side chain -2-^{14}C, bisuccinate) and 2·51 $\pm$ 0·07 for choline (methyl[^{3}H] chloride). In contrast, when the amines were replaced by amino-acids like L-3,4-dihydroxyphenylalanine (L-dopa) (side chain -3-^{14}C) and DL-5-hydroxytryptophan (-^{3}H, G) (DL-5HTP) (initial molar ratios of the amino-acid to ATP = 0·1), the establishment of a significant concentration gradient did not take place (values after 10 min for DL-5HTP: 1·12 $\pm$ 0·02, for L-dopa: 0·95 $\pm$ 0·04; n = 3–4), although the membrane was permeable to the amino-acids.

The establishment of an amine concentration gradient between chamber M_2 and M_1 might result from a reversible interaction of the amines with ATP in chamber M_2.

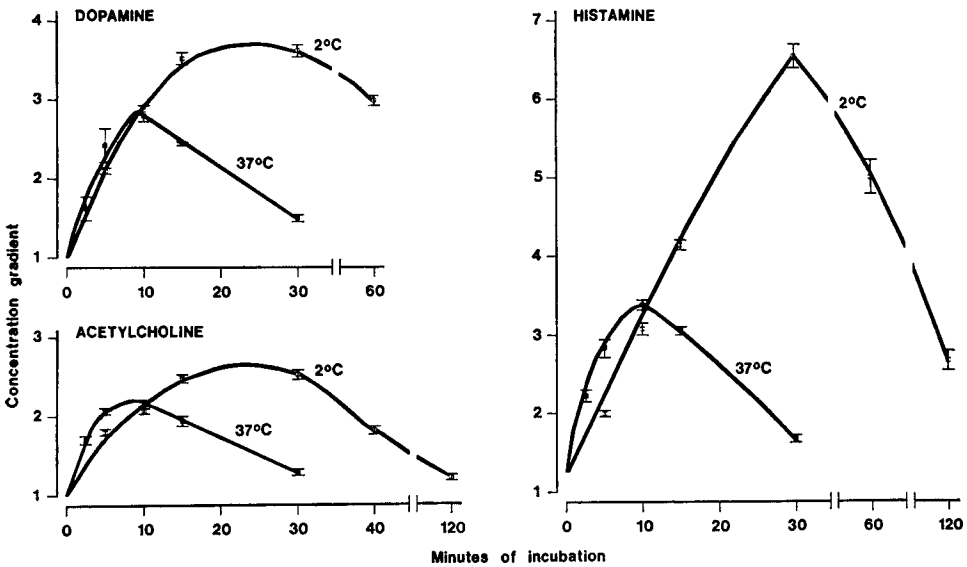

FIG. 2. Establishment of concentration gradients (molar concentration of amine in chamber M_2 divided by that in chamber M_1) of various amines in a microdiffusion system. The original amine concentration (at 0 min) in both chambers was equal (20 mM, concentration gradient = 1), whereas ATP originally was present in chamber M_2 only (200 mM). The points are average with s.e.m. of 7–8 experiments. The following radioactively labelled amines were used: dopamine (side chain -2-^{14}C, HCl), histamine (ring -2-^{14}C, dihydrochloride) and acetyl-1-^{14}C-choline chloride

Such an interaction, probably due to electrostatic and Van der Waals' forces, has been previously demonstrated for several aromatic monoamines using various physicochemical methods (Berneis, Da Prada & Pletscher, 1969a; Da Prada, Berneis & Pletscher, 1971; Pai & Maynert, 1972; Steffen, Da Prada & Pletscher, 1974; Weiner & Jardetzky, 1964). As a consequence of binding of the amines to ATP, the concentration of the free (diffusable) amine in chamber M_2 was decreased leading to a passage of the amine from chamber M_1 to M_2 and to the build-up of a concentration gradient between chamber M_2 and M_1. The decrease of this gradient after 10 min of incubation at 37° (Fig. 2) probably resulted from diffusion of ATP from chamber M_2 to M_1 which was shown to occur to a limited extent (presence of 27 and 53 μm ATP in chamber M_1 after 10 and 20 min respectively). The finding that the maximum amine concentration gradients attained at 2° were higher than at 37° was possibly due to a diminished passage of ATP from chamber M_2 to M_1 at lower temperature. In fact, 10 and 20 min after incubation at 2° only 6 and 13 mM respectively of ATP were present in chamber M_1.

From these *in vitro* experiments several conclusions regarding amine storage *in vivo* may be drawn. First, the findings confirm that amines can accumulate by mere physicochemical interaction against an apparent concentration gradient in a compartment containing ATP provided it is surrounded by a membrane impermeable to this nucleotide, but permeable to the amines. *In vivo*, these conditions seem to be fulfilled in a more ideal fashion than *in vitro* since, for instance, the granular membrane—in contrast to the artificial membrane—is virtually impermeable to ATP. This probably allows the build-up by physicochemical interaction of a much higher amine concentration gradient *in vivo* than in the present *in vitro* experiments. In addition, the molar concentration ratio between extracellular amine and intracellular ATP is probably very low *in vivo*. This may favour the establishment of high concentration ratios between extracellular and intracellular amines as seen in the above experiments with decreasing amine/ATP ratios.

Secondly, the present experiments showed that a concentration gradient was built up not only with aromatic monoamines but also with acetylcholine and its precursor choline. This indicates that acetylcholine and choline, too, interact with ATP. Interestingly, acetylcholine storage organelles from the electric organ of Torpedo have recently been found to contain a relatively high amount of ATP (Whittaker, Dowdall & Boyne, 1972), and this nucleotide was released from rat motor nerve terminals on electric nerve stimulation (Silinsky & Hubbard, 1973). It would seem, therefore, that the storage of acetylcholine and possibly of choline might also involve a reversible interaction with nucleotides such as ATP.

Thirdly, the maximum concentration gradients observed varied for the different amines, for instance, the gradient was higher with histamine, tryptamine and tyramine than with noradrenaline, dopamine and 5-hydroxytryptamine. However, the Mg/ATP-dependent amine uptake in isolated granular membranes of adrenal chromaffin granules showed a different order, the accumulation of dopamine, noradrenaline and 5-hydroxytryptamine being markedly superior to that of histamine, tryptamine and tyramine (Da Prada & others, 1975). It is therefore conceivable that the relative importance of the two mechanisms (intragranular interaction with ATP and ATP-dependent transport at the granular membrane level) in the storage of monoamines *in vivo* (e.g. in adrenal medulla) may differ for the various amines. The reason why in the experiments with histamine a higher concentration gradient resulted than in those with catecholamines and 5-hydroxytryptamine is being investigated now. The molecular size of the amine/ATP aggregates formed in chamber M_2 was probably not responsible since in previous experiments with analytical ultracentrifugation solutions of ATP plus histamine showed a smaller average apparent molecular weight than those of ATP plus catecholamines or 5-hydroxytryptamine (Berneis, Da Prada & Pletscher, 1969b,c).

In conclusion, the present experiments demonstrate that the accumulation of biogenic amines including acetylcholine in subcellular storage organelles is probably due not only to an active transport at the granular membrane level but also to a binding of the amines to intragranular ATP. Both the intragranular and the membrane mechanism seem to discriminate between the various amines with regard to their storage, the discrimination at the intragranular level being, however, different from that at the membrane level.

Research Department,
F. Hoffman-La Roche & Co. Ltd.,
Basle,
Switzerland.

M. Da Prada
R. Obrist
A. Pletscher

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REFERENCES

Berneis, K. H., Da Prada, M. & Pletscher, A. (1969a). *Science*, **165**, 913–914.

Berneis, K. H., Da Prada, M. & Pletscher, A. (1969b). *Agents Actions*, **1**, 35–38.

Berneis, K. H., Pletscher, A. & Da Prada, M. (1969c). *Nature*, **224**, 281–283.

Berneis, K. H., Da Prada, M. & Pletscher, A. (1974). *Ibid.*, **248**, 604–606.

Da Prada, M., Berneis, K. H. & Pletscher, A. (1971). *Life Sci.*, **10**, 639–646.

Da Prada, M., Obrist, R. & Pletscher, A. (1975). *Br. J. Pharmac.*, in the press.

Pai, V. S. & Maynert, E. W. (1972). *Mol. Pharmac.*, **8**, 82–87.

Pletscher, A., Da Prada, M., Berneis, K. H., Steffen, H., Lütold, B. & Weder, H. G. (1974). In: *Advances in Cytopharmacology*, vol. 2, pp. 257–264. Editors: Ceccarelli, B., Clementi, F. & Meldolesi J. New York: Raven Press.

Silinsky, E. M. & Hubbard, J. I. (1973). *Nature*, **243**, 404–405.

Steffen, H., Da Prada, M. & Pletscher, A. (1974). *Biochim. biophys. Acta*, **338**, 561–571.

Weiner, N. & Jardetzky, O. (1964). *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **248**, 308–318.

Whittaker, V. P., Dowdall, M. J. & Boyne, A. F. (1972). *Biochem. Soc. Symp.*, **36**, 49–68.