

was added in amounts shown in the figure. The transmittance changes in the actomyosin suspension upon the addition of ATP was done in  $1 \times 1$  cm glass cell of 4 cm height in the optical path of Beckman DU2-spectrophotometer at  $660 \mu\text{m}$  wavelength and  $27^\circ\text{C}$ .

**Results and Discussion.** Variations in the ATP concentration and their effect on actomyosin superprecipitation and development of tension by glycerinated fibres have been studied. At the low ATP concentration, both parameters are limited by the availability of ATP. That is, the addition of more ATP increased both the magnitude and the rate of superprecipitation and the development of tension by glycerinated fibers. At high substrate levels, the phenomenon of excess substrate inhibition occurs. Both superprecipitation and tension development are inhibited by the addition of excess substrate. In Figure 1 curve a) shows the variations in ATP concentrations and their effect on actomyosin superprecipitation, curve b) shows the effect of 0.25 mg protein of the serum enzyme in the assay system.

Addition of cyclic 3', 5'-AMP into the assay system affected actomyosin superprecipitation and development of tension by glycerinated muscle fibers in a comparable manner. If glycerinated fibers are incubated with cyclic 3', 5'-AMP for 5 min before the addition of ATP, the contraction of the fibers is inhibited to a degree dependent on the concentration of the cyclic nucleotide. Figure 2 shows the concentration dependence of the cyclic 3', 5'-AMP inhibition of the development of tension at 0.05, 0.5, 1.2 and  $1.5 \text{ mM}$  ATP concentrations. The data suggest that the inhibitory effect of the cyclic nucleotide is complex and varies inversely with ATP concentration.

If glycerinated fibers are incubated first with the serum enzyme and then cyclic 3', 5'-AMP is added and incubation continued for 5 min before the addition of ATP, the contraction of the fiber is inhibited to a degree dependent on the concentration of both the serum enzyme and the cyclic nucleotide. When no serum enzyme is added (curve a, Figure 3) the contraction of the fiber is inhibited

to a degree dependent on the concentration of the cyclic 3', 5'-AMP at  $0.05 \text{ mM}$  ATP. The effect of 0.10, 0.25 and 0.50 mg protein of the serum enzyme on the cyclic 3', 5'-AMP inhibition of the development of tension at  $0.05 \text{ mM}$  ATP is shown by curves b, c, and d respectively.

The upper tracing a) of Figure 4 shows the response of a glycerinated fiber preincubated in  $\text{Mg}^{++}$ -deficient *tris*-phosphate buffer containing the serum enzyme. Weak tension developed slowly after addition of ATP. If  $\text{Mg}^{++}$  was then added, full tension developed. Additional amounts of ATP led to development of maximum tension.

Tracing b) of Figure 4 shows the response by glycerinated fibers preincubated in the  $\text{Mg}^{++}$ -deficient *tris*-phosphate containing cyclic 3', 5'-AMP. Addition of ATP caused development of a very weak tension. The magnitude of tension induced by addition of magnesium was approximately  $1/4$  that produced in tracing a).

Tracing c) shows the response of glycerinated fibers preincubated in the  $\text{Mg}^{++}$ -deficient *tris*-phosphate to which cyclic 3', 5'-AMP and the serum enzyme were added. Addition of ATP produced an enhanced development of tension. Addition of  $\text{Mg}^{++}$  resulted in further development of tension.

**Résumé.** Les effets d'une enzyme du sérum humain sur la contractilité de la fibre musculaire glycerinée, et sur la superprécipitation de l'actomyosine sont démontrés. Les effets produits sur l'inhibition par excès du substrat et sur l'inhibition par l'adénosine 3', 5'-monophosphate cyclique des deux paramètres, suggèrent que l'enzyme du sérum est un facteur qui contrôle la contraction musculaire.

A. A. HAKIM

*Biochemical Research Laboratories of the I.T.R.,  
259 D.M.P. School of Medicine,  
University of Illinois at the Medical Center,  
Chicago (Illinois 60680, USA), 31 August 1970.*

## The Effect of 5-Hydroxytryptophan on the Efflux of Noradrenaline from Brain Slices

Recently the possibility of reciprocally acting 'catecholaminergic' and 'serotonergic' systems in the brain has been proposed<sup>1,2</sup>, whereby not the absolute level of a given amine in a given mechanism (e.g. sleep<sup>3</sup>, aggression<sup>4,5</sup>, motor activity<sup>6</sup>, psychoses<sup>7</sup>) is important, but the relative levels of available transmitter.

Starting from this suggestion, we have compared the effects of different additives on the efflux of noradrenaline (NA) from brain slices. Two systems were studied: efflux of exogenous NA previously taken up from the incubation medium, and - with the possibility in mind that a part of the uptake may not be specifically localised - efflux of NA newly synthesized from L-DOPA.

**Experimental.** The di- and mesencephalon ('mid-brain') from male Füllinsdorf albino rats were sliced<sup>8</sup> and preincubated for 30 min in Krebs-Ringer bicarbonate glucose supplemented medium containing pyridoxal phosphate ( $10^{-5} \text{ M}$ )<sup>9</sup>, under an atmosphere of 95%  $\text{O}_2$ -5%  $\text{CO}_2$ , either with NA or DOPA (both  $10^{-3} \text{ M}$ ). The tissues were washed and resuspended in an incubation medium containing the compound under investigation, and aliquots of tissue were taken at intervals. After perchloric acid extraction of the tissue, NA was determined after alumina absorption by a modified fluorescence method<sup>10</sup>, seroto-

nin (5-HT) also by fluorescence<sup>11</sup>; protein was determined in the precipitate<sup>12</sup>.

**Results.** NA was readily taken up or synthesized from DOPA. After washing, the level of NA in the tissue

<sup>1</sup> H. TAKAGI, M. SATOH, K. YAMATSU, K. KIMURA and M. NAKAMA, *Int. J. Neuropharmac.* 7, 265 (1968).

<sup>2</sup> F. P. MILLER and R. P. MAICKEL, *Life Sci.* 8, 487 (1969).

<sup>3</sup> M. JOUVET, *Science* 163, 32 (1969).

<sup>4</sup> E. LYCKE, K. MODIGH and B. E. ROOS, *Experientia* 25, 951 (1969).

<sup>5</sup> H. LAL, J. J. DEFEQ and P. THUT, *Pharmacologist* 11, 278 (1969).

<sup>6</sup> T. L. CHRUSCIEL and Z. S. HERMAN, *Psychopharmacologia* 14, 124 (1969).

<sup>7</sup> H. C. STANCER, B. QUARRINGTON, B. A. COOKSON and G. M. BROWN, *Arch. gen. Psychiat.* 20, 290 (1969).

<sup>8</sup> H. MCLWAIN and R. RODNIGHT, *Practical Neurochemistry* (Churchill, London 1962).

<sup>9</sup> S. F. CONTRACTOR and M. K. JEACOCK, *Biochem. Pharmac.* 16, 1981 (1967).

<sup>10</sup> U. S. VON EULER and F. LISHAJKO, *Acta physiol. scand.* 51, 348 (1961).

<sup>11</sup> S. SNYDER, J. AXELROD and M. ZWEIG, *Biochem. Pharmac.* 14, 831 (1965).

<sup>12</sup> O. L. LOWRY, J. N. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. biol. Chem.* 193, 265 (1951).

decreased at a constant rate over the period of 30–60 min. The concentration of NA remaining in the tissue was of the same order for newly synthesized NA and NA uptake.

1. 5HT ( $10^{-3} M$ ) in the incubation medium had no effect on the rate of NA efflux in either system.

2. The precursor of 5HT, 5-hydroxytryptophan (5HTP,  $10^{-3} M$ ), had no effect on the rate of efflux of NA previously taken up from the medium (Figure 1). In contrast, 5HTP lowered the levels of NA previously synthesized from DOPA to ca. 40% control values. The difference from control values was significant at the 0,01 level (rank summation), except for the last values (75 min) (Figure 2).

3. The amino-acids histidine and L-tryptophan caused the same decrease of NA in tissue, whereas the corresponding amines had no effect. As with 5HTP, the effect

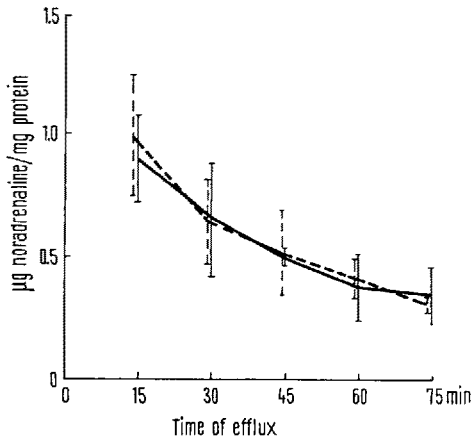


Fig. 1. Noradrenaline uptake: effect of 5HTP on efflux.

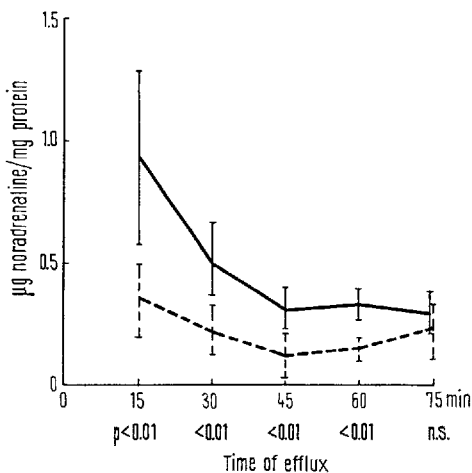


Fig. 2. Noradrenaline synthesis: effect of 5HTP on efflux.

was only found with NA synthesised from DOPA, not with uptake NA.

4. The decarboxylase inhibitor Ro 4-4602 in the incubation medium also resulted in a significant lowering of NA levels in the tissue, but only after 30 min. The effect of the dopamin-hydroxylase inhibitor disulfiram was apparent in a lowering of NA levels after 45 min.

*Discussion.* The mechanism of depletion of brain tissue NA levels by 5HTP appears to be by inhibition of further NA synthesis from DOPA, rather than by a 5HTP (or 5HT) mediated efflux. This effect has been reported in vivo<sup>13</sup>, where the lowered NA levels after doses of 5HTP causing overt stimulation in the rat were attributed to inhibited synthesis and not to stimulated release of NA<sup>14</sup>.

5HTP (as histidine and L-tryptophan) utilizes the same unspecific enzyme as DOPA in its synthetic pathway: 1-aromatic-amino-acid decarboxylase<sup>15</sup>. These substrates can act as competitive inhibitors.

Preincubation of brain slices simultaneously with DOPA and 5HTP results in mutual inhibition of synthesis of the amines (75% of control value,  $p < 0.01$ ). The rate of efflux of both amines is not significantly different from control values.

A common enzyme for the decarboxylation of the different amino-acid precursors of the biogenic amines may indicate that the first step of NA biosynthesis (hydroxylation) may not be the only limiting factor of amine formation. Changes of cerebral pyridoxal phosphate levels may also be important for feedback mechanisms involving pyridoxal phosphate dependent enzymes<sup>9,16</sup>. An overload of any one aromatic amino acid could have implications for the regulated balance of amines in the brain<sup>17</sup>.

*Zusammenfassung.* 5-Hydroxytryptophan erniedrigt die Konzentration von Noradrenalin in Hirnschnitten, sofern diese das Amin zuvor aus zugesetztem L-DOPA selber synthetisierten. Dieser Effekt beruht vermutlich darauf, dass 5-Hydroxytryptophan die Decarboxylierung des L-DOPA, das im Hirngewebe gespeichert wird, kompetitiv hemmt. Die Relation der Noradrenalin- und Serotoninkonzentration im Hirngewebe kann somit auch durch ein verändertes Angebot an Aminosäuren gestört werden.

H. FEER and A. WIRZ-JUSTICE

Biochemisches Laboratorium der Psychiatrischen  
Universitätsklinik Basel, CH-4056 Basel (Switzerland),  
8 February 1971.

<sup>13</sup> G. A. JOHNSON, E. G. KIM and S. J. BOUKMA, Proc. Soc. exp. Biol. Med. 128, 1948 (1968).

<sup>14</sup> B. B. BRODIE, M. S. COMER, E. COSTA and A. DLABAC, J. Pharmac. exp. Ther. 152, 340 (1966).

<sup>15</sup> P. M. CEASAR, B. F. ANAGNOSTE and M. GOLDSTEIN, Abstr. Papers, Am. chem. Soc. 160, 102 (1970).

<sup>16</sup> J. A. BUZARD and P. D. NYTCH, J. biol. Chem. 227, 225 (1957).

<sup>17</sup> S. R. TONGE and B. E. LEONARD, Life Sci. 9, 1327 (1970).

## Leichtflüchtige Stoffe in *Agaricus bisporus*

Es ist bekannt, dass unter den äusseren Faktoren, welche Wachstum, Fruktifikation, Morphogenese und Sporenkeimung von Pilzen beeinflussen, neben den Feuchtigkeits-, Licht- und Temperaturverhältnissen der Anwesenheit von leichtflüchtigen Stoffen – grösstenteils unbekannter Identität – oft eine entscheidende Rolle zukommt. Es

dürfte sich dabei in den meisten Fällen um niedere Fettsäuren, Fettsäureester, niedere Alkohole, Aldehyde und  $CO_2$  handeln. So stimulieren Vertreter der erstgenannten Stoffgruppe ( $C_1 - C_6$ ) die Sporenkeimung von *Phycomyces*<sup>1</sup>, Ameisensäure aktiviert die Pseudophorenbildung bei *Rhizopus sexualis*<sup>2</sup>, und Isovaleriansäure fördert die Keimung