Versuchsanordnung. In die Reagensgläser wurden folgende Lösungen pipettiert:

0,1 ml RP-Serum
0–0,4 ml Inhibitor
0,4–0 ml Puffer pH 7,4.

Das Volumen des Systems betrug immer 0,5 ml, die Konzentration des Inhibitors variierte von 0 bis 1,6 g%. Die Lösung wurde während 15 min bei 37°C inkubiert; dann wurden 2 ml Erythrozytensuspension zugefügt und das Gemisch weitere 30 min bei gleicher Temperatur gehalten. Nach der Inkubation wurde die Suspension zentrifugiert und photometrisch (Unicam) die Extinktion gemessen. Eine Hämolyse ohne Inhibitorzugabe wurde als 100% angenommen (Abb. 1).

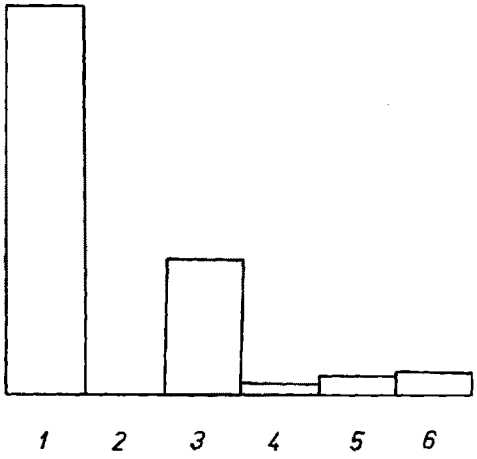


Abb. 4. Relative Hemmungswirkung von Antiproteasen auf Plasmin. Der Inhibitionseffekt der Antiprotease aus Pankreas wurde als Basis genommen und die Wirkung der übrigen Inhibitoren im Verhältnis zum Pankreasinhibitor bestimmt. *1* = Inhibitor aus Pankreas, *2* = Ovomucoid, *3* = aus Sojabohnen, *4* = aus Phaseolus, *5* = aus Kartoffeln, *6* = IV. Fraktion von Humanplasma.

Die Antiproteasewirkung auf das hämolytische System äusserte sich in einer Hemmung der prozentualen Lyse, wie Abbildung 1 und 2 zeigen. Die intensivste Wirkung besass der aus Sojabohnen hergestellte Inhibitor, während die aus Pankreas, Phaseolus und Kartoffeln gewonnenen Präparate eine relativ geringere Hemmung ergaben. Ovomucoid war ohne jeglichen Effekt. Die native IV. Plasmafraktion zeigte eine Properdin-hemmende Wirkung, die nach Erwärmung auf 60°C allmählich verschwand.

Die hemmende Wirkung von Properdin auf C'_3 in Gegenwart von Zymosan bei 37°C wurde durch die Inhibitoren mit Ausnahme der IV. Fraktion nicht gestört; die resultierende Hämolyseverminderung setzte sich aus der inaktivierenden Wirkung des Properdins und der Komplementhemmung durch die Antiproteasen zusammen. Bei Inhibitoren aus Sojabohnen und Kartoffeln überstieg die aktuelle Inhibition die Summe beider Inaktivierungswirkungen (Abb. 2).

Die hier beobachtete Komplement-Hemmung durch Antiproteasen liefert einen weiteren Beweis für den Proteasencharakter mindestens einer Komplementkomponente (C'_1). Wir sind ferner der Ansicht, dass dieser Inhibitionseffekt in der Therapie einiger allergischer Erkrankungen und vielleicht auch der toxischen hämolytischen Anämien ausgenützt werden könnte.

M. RYBAK, M. PETAKOVA, V. MANSFELD, and J. HLADOVEC

Institut für Hämatologie und Bluttransfusion und Forschungsinstitut für Pharmazie und Biochemie, Prag, 7. Mai 1959.

Summary

The complement-inhibiting action of some antiproteases (i.e. the inhibitors from pancreas, sojabeans, Phaseolus, potatoes, ovomucoid, and the effect of the IV. ethanol plasma fraction) has been observed. It was found that the antiproteases produced from sojabeans, pancreas, Phaseolus, and potatoes possess the inhibitory effect.

On the Distribution in Brain of Monoamines and of Enzymes Responsible for their Formation

In a previous investigation, the distribution of noradrenaline and dopamine in the brains of several mammalian species was mapped out[1,2]. High amounts of noradrenaline were detected in the hypothalamus and other parts of the brain stem, whereas dopamine was found to be localized almost exclusively in the nuclei of the corpus striatum of the cerebral hemispheres. The brain parts rich in catechol amines also proved to have high L-dihydroxyphenylalanine (DOPA) decarboxylase

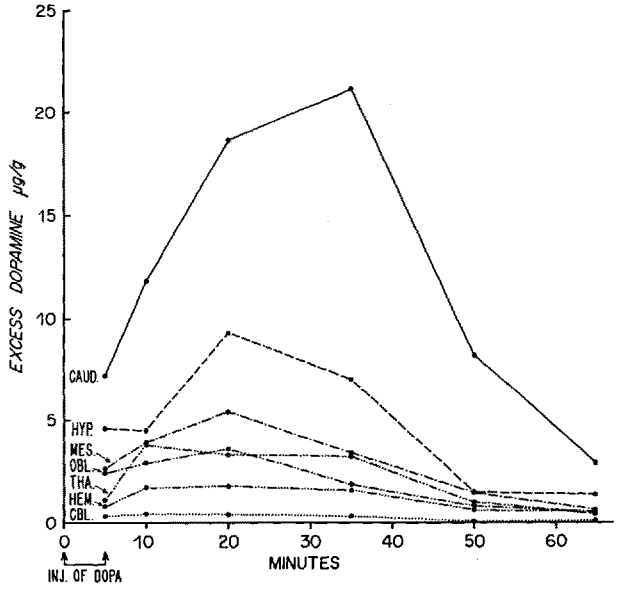


Fig. 1. – Excess concentrations of dopamine in different brain parts at varying intervals of time after injection of DOPA. Caud.: Caudate nucleus. Hyp.: Hypothalamus. Mes.: Mesencephalon. Obl.: Medulla oblongata + pons. Tha.: Diencephalon (not hypothalamus). Hem.: Hemispheres (not corpus striatum). Cbl.: Cerebellum

activities *in vitro*. These and other data seemed to indicate that the two amines were involved in the functions of different regions of the brain. The investigation has now been continued and some additional data are given in this paper.

It was thought of interest to study the distribution of noradrenaline and dopamine in some further detail. The

[1] Å. BERTLER and E. ROSENGREN, Exper. *15*, 10 (1959).

[2] Å. BERTLER and E. ROSENGREN, Acta physiol. Scand. (in press).

human brain seemed well suited for such studies, as the various structures of this brain can be more easily dissected out than in other species. The brain tissues were obtained from the post mortem room 6–16 h after death of the patients. They were examined for their noradrenaline and dopamine contents as described in earlier papers[2–4].

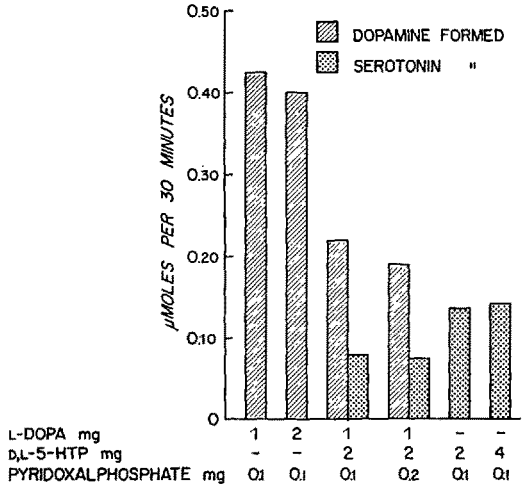


Fig. 2. – Decarboxylation of DOPA and 5-HTP and a mixture of the two amino acids by a hog brain extract. Each vessel contained 2·5 ml hog brain extract (pH 7·5) corresponding to 0·7 g tissue. Temperature 37° C. Final volume 3·1 ml

Considerable amounts of dopamine were found in the caudate nucleus and the putamen, which contained 2·0 to 6·5 µg/g. In the globus pallidus no dopamine could be detected. The highest concentration (0·8–1·9 µg/g) of noradrenaline in the human brain was observed in the rostral and intermediate portions of the hypothalamus. Lower values were recorded in the region of the mammillary bodies. The noradrenaline and dopamine values in these and other areas of human brain were about 30–50% of those observed in other species. The lower values in the human brain may be explained by the long interval between death and extraction of the tissues. It is interesting that dopamine is confined to cytologically similar nuclei which constitute the phylogenetically youngest part of the corpus striatum. Noradrenaline and 5-hydroxytryptamine (5-HT) are localized mainly in older areas of the brain.

The uneven distribution of catechol amines in brain has prompted a study of the capacity of different brain parts to form and inactivate such amines. To this end DOPA, which is the precursor of dopamine, was injected intravenously into normal adult rabbits in a dose of 100 mg DL-form/kg. The duration of injection was 5 min. The animals were killed at varying intervals of time after the end of the injection by a blow on the neck and the blood was collected. The brains were divided into the various parts and the corresponding parts from two animals analyzed together. The results are given in Figure 1. It will be seen that after administration of DOPA, dopamine accumulated in the brain, which is in accordance with earlier observations[5]. The increase varied, however, widely from one part of the brain to another. The rate of accumulation roughly parallelled the concentration of preformed catechol amines, i.e. it was highest in the caudate nucleus and the hypothalamus, somewhat lower in the rest of the brain stem, and still lower in the cerebral hemispheres and the cerebellum. The excess concentrations of dopamine reached their maxima 15–30 min after the end of the DOPA injection and then decreased at rates suggesting a first order reaction. When plotted on a semilogarithmic paper, the slopes of the declining parts of the curves were practically the same for the different parts of the brain. The ability to destroy dopamine thus appears to be the same throughout the brain, in agreement with the fact that the distribution of monoamine oxidase in brain is fairly uniform[6].

In a similar manner we have studied the accumulation of 5-HT after the injection of 5-hydroxytryptophan (5-HTP). The highest values of excess 5-HT were found in the hypothalamus and the caudate nucleus, the lowest in the cerebral hemispheres and the cerebellum[7].

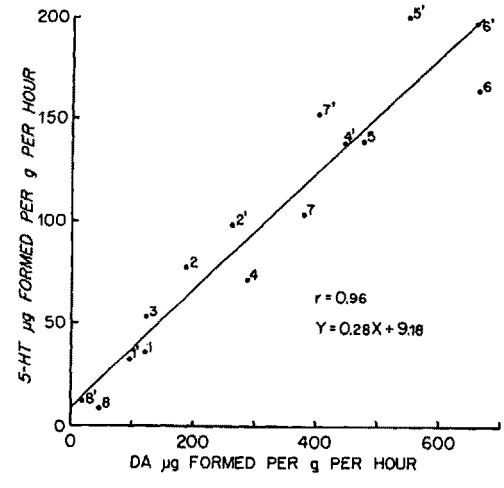


Fig. 3. – 5-HTP vs. DOPA decarboxylase activities in different parts of rabbit brain.

1. Hemispheres (not corpus striatum); 2. Caudate nucleus; 3. Lentiform nucleus; 4. Diencephalon (not hypothalamus); 5. Hypothalamus; 6. Mesencephalon; 7. Medulla oblongata + Pons; 8. Cerebellum. 1 and 1′ etc. represent data from two different experiments

The interpretation of the above data is partly dependent on the specificity of the DOPA and 5-HTP decarboxylases, a problem which has not yet been settled[9–11]. We have observed that DOPA decarboxylase in hog kidney cortex extracts was competitively inhibited by 5-HTP

[3] Å. Bertler, A. Carlsson, and E. Rosengren, Acta physiol. Scand. *44*, 273 (1958).

[4] A. Carlsson and B. Waldeck, Acta physiol. Scand. *44*, 293 (1958).

[5] A. Carlsson, M. Lindqvist, T. Magnusson, and B. Waldeck, Science *127*, 471 (1958).

[6] S. Udenfriend, P. A. Shore, D. F. Bogdanski, H. Weissbach, and B. B. Brodie, Recent Progr. Hormone Res. *13*, 1 (1957).

[7] In these experiments the examination of the different brain parts for their 5-HT contents was performed with a procedure which has been in use in this laboratory. The extraction and purification was principally carried out as described for catechol amines[3], except that another type of ion exchange resin was used, Amberlite XE-64 instead of Dowex 50. The final determination was performed fluorimetrically as described by Udenfriend[8].

[8] S. Udenfriend, H. Weissbach, and D. F. Bogdanski, Science *122*, 972 (1955).

[9] C. T. Clark, H. Weissbach, and S. Udenfriend, J. biol. Chem. *210*, 139 (1954).

[10] E. Westermann, H. Balzer, and J. Knell, Arch. exp. Path. Pharmak. *234*, 194 (1958).

[11] A. Yuwiler, E. Geller, and S. Eiduson, Arch. Biochem. Biophys. *80*, 162 (1959).

and *vice versa*. In other words, the data suggested that DOPA and 5-HTP were competing for one and the same enzyme. Experiments performed with hog brain extracts gave similar results. In Figure 2, the data from a typical experiment are given, in which an extract from the caudate and lentiform nuclei, the diencephalon and the mesencephalon was used. It will be seen that the addition of one of the amino acids caused an inhibition of the decarboxylation of the other. The inhibition was 50% when the concentrations of the L-forms of the two amino acids were equal. The inhibition does not seem to be due to lack of co-enzyme, as doubling the amount of pyridoxal-5-phosphate had no effect on the results.

A similar competition possibly also occurs *in vivo*: Repeated injections to rabbits of DOPA (600 mg/kg in total) during 2 h lowered the brain 5-HT level to 50–60% of the normal. Whether this decrease was due to inhibited biosynthesis or to release followed by oxidation, remains to be discovered, however.

In order further to elucidate this problem, DOPA and 5-HTP decarboxylase activities in various regions have been determined using the same technique as described earlier[2]. The results are given in Figure 3. A certain degree of correlation between the initial accumulation of dopamine after DOPA injection and DOPA decarboxylase activity is apparent from a comparison of Figures 1 and 3. A high order of correlation was found between the distribution of the two amino acid decarboxylases. This finding lends additional support to the assumption that the decarboxylation of the two amino acids is carried out by one and the same enzyme.

After the injection of DOPA, the animals showed increased motor activity and signs of sympathetic stimulation. The effect on motor activity appeared to be closely correlated to the accumulation of dopamine in brain: both phenomena appeared to reach their maxima in about 25 min. Motor activity then appeared to decline along with the drop in the dopamine level. About 50 min. after the injection of DOPA, there was still definite evidence of central excitation. It is interesting to note that at this interval the dopamine levels were fairly low in all parts of the brain except the corpus striatum, suggesting that this part of the brain forms an important site of action of dopamine on motor functions. Administration of 5-HTP caused an appearance quite different from that produced by DOPA. There was no motor hyperactivity, rather a decrease in voluntary movements combined with general muscle tremors.

This work has been supported by grants from the Swedish Medical Research Council and by the Air Force Office of Scientific Research of the Air Research and Development Command, United States Air Force.

Å. BERTLER and E. ROSENGREN

Department of Pharmacology, University of Lund (Sweden), July 24, 1959.

Zusammenfassung

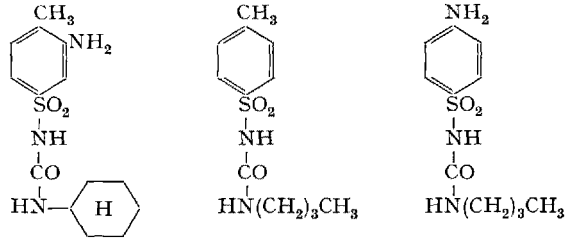
Im menschlichen Gehirn kommen grosse Mengen von 3-Hydroxytyramin im Nucleus Caudatus und dem Putamen vor.

Injektion von 3,4-Dihydroxyphenylalanin in Kaninchen bringt eine schnelle Akkumulation von 3-Hydroxytyramin in den Gehirnteilen, die reich an Katecholaminen sind, zustande. Die Fähigkeit das akkumulierte 3-Hydroxytyramin abzubauen scheint im ganzen Gehirn dieselbe zu sein.

Hemmungsversuche und Aktivitätsuntersuchungen der Fermente in verschiedenen Gehirnteilen *in vitro* sprechen dafür, dass 5-Hydroxytryptophan und 3,4-Dihydroxyphenylalanin von einem und demselben Ferment decarboxyliert werden.

On the Hypoglycemizing Effect of Metahexamide

Metahexamide (N(3-amino-4-methyl-benzenesulfonyl)-N'-cyclohexylurea) is a new oral hypoglycemizing agent[1] (see formula) having a better therapeutic index than other similar drugs.



Metahexamide D 860 BZ 55

Following the line of our previous investigations we have been interested to study the hypoglycemic action, the absorption and disappearance from blood and the effect on glucose uptake by rat diaphragm *in vitro*.

Methods. Metahexamide was always administered by stomach tube, as the reference drug *p*-tolylsulfonilbutylurea (D 860). Blood sugar levels were determined in duplicate in 0·1 ml samples by the Nelson procedure[2] in 12 h fasted Sprague-Dawley rats or rabbits of mixed breed.

Metahexamide has been evaluated in blood and kidney with a modified method of BRATTON and MARSHALL[3]. To the filtrate obtained after precipitation with 15% trichloroacetic acid and filtration through no. 1 Whatman filter paper, were added 0·1% sodium nitrite (1 ml), 0·5% ammonium sulfamate (1 ml), concentrated hydrochloric acid (1 ml) and 0·1% N (1-naphthyl)ethylenediamine dihydrochloride (1 ml). The resulting colour was read in a photoelectric colorimeter using a filter having a maximum transmission at 520 mμ. Glucose uptake tests were performed on rat hemidiaphragms in Krebs-Ringer bicarbonate buffer as described elsewhere[4–6].

Results

a) *Hypoglycemic activity in vivo.* Metahexamide and D 860 (as reference drug) have been found to be active oral hypoglycemizing drugs both in rats and rabbits. In rats Metahexamide induces maximum blood glucose falls of 20, 35, and 50% respectively after 10, 40, and 100 mg/kg[7], while D 860 is without activity at 10 mg/kg and at 50 and 100 mg/kg, produces falls of blood sugar level to 35 and 50% respectively.

The maximum effect is achieved for both drugs about 2 h after administration, but Metahexamide hypoglycemia

[1] D. MÜTING, W. PRESSER, and K. SHIVAROM, Arzn.Forsch. *9*, 188 (1959).
[2] N. NELSON, J. biol. Chem. *153*, 375 (1944).
[3] A. BRATTON and E. MARSHALL, J. biol. Chem. *128*, 537 (1939).
[4] C. R. PARK *et al.*, Amer. J. Physiol. *182*, 12 (1955).
[5] N. CANAL *et al.*, Clin. Terap. *11*, 472 (1956).
[6] S. GARATTINI *et al.*, Arzn.Forsch. *8*, 477 (1958).
[7] The LD_{50} of Metahexamide by oral route in rat is about 2 g/kg.