Nach Iproniazid-Applikation hatten nicht nur 5HT und Adrenalin, sondern auch andere Monoamine (Phenyläthylamin, Tyramin, Mezcalin) verstärkte temperatursteigernde Wirkung. Durch Hemmung der Monoaminoxydase fällt möglicherweise ein allgemeiner Schutzmechanismus des ZNS gegen die Einwirkung von Monoaminen aus. Die Befunde bei kombinierter Iproniazid/Reserpin-Applikation lassen daran denken, dass endogene Monoamine (zum Beispiel 5HT, Adrenalin, Noradrenalin) bei gestörter Aktivität der Monoaminoxydase erregende Wirkung auf das ZNS ausüben können.

H. Bächtold und A. Pletscher

Medizinische Forschungsabteilung der F. Hoffmann-La Roche & Co., AG., Basel, den 31. Januar 1957.

Summary

The influence of isopropyl-isonicotinic acid hydrazide (Iproniazid) on the course of body temperature after reserpine, 5HT, adrenaline, various other monoamines, as well as chlorpromazine, was investigated in rabbits and compared with the temperature effect of lysergic acid diethylamide. Furthermore, the influence of isonicotinic acid hydrazide (Isoniazid) on the temperature effect of reserpine and 5-hydroxytryptamine (5HT) was measured. The following results were obtained:

(1) After pretreatment with Iproniazid, reserpine no longer produced hypothermia, but marked hyperthermia.

(2) The hyperthermic effect of 5HT and various other monoamines was significantly increased by Iproniazid-pretreatment.

(3) Isoniazid-pretreatment did not influence the temperature effect of reserpine and 5HT.

(4) The hypothermic effect of chlorpromazine was not significantly changed by Iproniazid-pretreatment.

DISPUTANDUM

Effects of Pyrithiamine and Oxythiamine on the Thiamine Content of Tissues and Blood Pyruvate in Mice

De Caro *et al.* have stated in their paper in Experientia *12*, 300 (1956) that Woolley and Merrifield based their evidence on the fact that pyrithiamine did not decrease the thiamine content of tissues. This statement is not true. Woolley and Merrified showed that the cocarboxylase content of tissues and the pyruvate content of blood were not reduced by pyrithiamine. Actually they feel very strongly that pyrithiamine should reduce the *thiamine* content of tissues. The fundamental mode of action of an antimetabolite is to displace the related metabolite and thus to lower the content of this metabolite in the tissues. In the case of pyrithiamine then, one would expect the thiamine content of the tissues to be lowered.

The whole point of Woolley and Merrifield's argument was not that the thiamine content was unchanged, but rather that the cocarboxylase content was not changed. Consequently, the demonstration by De Caro *et al.* that the thiamine content of brain is decreased in animals previously treated with pyrithiamine does not differ from the concept of Woolley and Merrifield and most certainly cannot be taken as evidence that the view of the latter authors with respect to a new function for thiamine is erroneous.

D. W. Woolley

We think that in our paper the essential points of Woolley and Merrifield hypothesis are clearly exposed, the importance of cocarboxylase content of the tissues being sufficiently emphasized.

In fact exposing our experimental data we clearly referred to the total thiamine content, once in the text and once in the table. The term 'total thiamine' cannot be misleading: as is well known, it means free thiamine + phosphorylated thiamine (cocarboxylase). After the indications in the text and in the table, we spoke often of thiamine, but, obviously, always in the sense of total thiamine: we did not consider it necessary to repeat it every time.

Further, it is well known that the thiamine in the tissues is nearly all in the form of cocarboxylase, especially in the nervous and muscular tissues.

In particular in normal mice brain, we recently found the following results:

total thiamine μg 3·16/g
free thiamine μg 0·05/g

Hence it is clear that a significant decrease of the total thiamine can only be a consequence of a significant decrease of the cocarboxylase. Now, it is out the question that the Pyrithiamine sharply lowers (from 2·16 to 0·74 μg/g) the total thiamine content and, therefore, the cocarboxylase content in the mice brain.

In our recent paper [Intern. Z. Vitaminforsch. *26*, 343 (1956)] on the rats (treated by mg 2·5 of Pyrithiamine for 6 days), we were able to show the behaviour of free and phosphorylated thiamine (or cocarboxylase). Actually the Pyrithiamine lowered, in these experiments, the brain phosphorylated thiamine from 2·8 μg/g to 0·72 μg/g, while the free thiamine decreased from 0·08 μg/g to 0·02 μg/g. In the muscle and in the liver too, the Pyrithiamine lowered the phosphorylated thiamine content.

Therefore we still consider our conclusion right that the Woolley and Merrifield hypothesis, based on the claim that the Pyrithiamine does not modify the cocarboxylase content of the tissues, is not experimentally sustainable.

L. De Caro, G. Rindi, V. Perri, and G. Ferrari

Allow me to say once again that De Caro *et al.* have not stated the findings of Woolley and Merrifield correctly. Their findings about thiamine content of tissues are *not* contradictory to the observations of Woolley and Merrifield and consequently they are unjustified in stating in your journal that they have been unable to confirm the experimental findings on which Woolley and Merrifield based their conclusions. Woolley and Merrifield made their measurements of cocarboxylase. De Caro, Rindi, Perri, and Ferrari are consequently unjustified in stating in their paper that Woolley and Merrifield made measurements on thiamine. Thiamine is not the same substance as cocarboxylase.

Although it is widely believed that much of the thiamine in normal tissues is present in a combined form, it

is by no means proven that this is the case in animals treated with pyrithiamine and in fact it is not accepted by all investigators that all of the combined thiamine is identical with cocarboxylase. Consequently, the reply of DE CARO, RINDI, PERRI, and FERRARI to my previous objection is not convincing.

D. W. WOOLLEY

With this I declare: End of the discussion. The Redactor.

PRO EXPERIMENTIS

Detection of Dipeptides and Dipeptidase Activity on Paper

During the course of some work[1] on the purification of pig kidney cysteinyl-glycinase[2], the usefulness of working out a quick method to detect the dipeptidase activity became apparent.

The method is a modification of GIRI and NAGABHUSHANAN'S test for the detection of amino acids on paper[3], and is based on the observation that, after spraying with naphtoquinonesulfonate and during the following alcohol-alkali treatment, some dipeptides change their colour at a lower rate or develop a different colour than the corresponding amino acids, thus enabling the use of this reaction for the detection of dipeptidase activity.

Procedure. Reagent *a:* 0·3 g of Na β-naphtoquinone-4-sulfonate (Eastman) is dissolved in 10 ml water, and distilled acetone is added to 100 ml. The reagent must be prepared immediately before use.

Reagent *b:* 2 ml of 4 *N* NaOH is diluted to 100 ml with 95% ethanol.

The paper is sprayed with reagent *a*, and heated in an oven at 100°C for 3–5 min. At this stage, amino acids and dipeptides develop colours which are often different enough to permit one to distinguish between them: cysteinyl-glycine, e.g., gives a pink-yellow colour; glycine and cysteine a violet one.

A better differentiation is obtained if the paper is then dipped in a bath of reagent *b*. The colours of most of the amino acids turn to grey-green within 10–15 min, whilst most of the dipeptides tested retain for a longer time the staining acquired during the heating, or develop a different colour. After some hours' bath, the contrast is much less clear.

Colours of some dipeptides and of the corresponding amino acids after 10 min treatment with alcohol-alkali.

Glycyl-glycine	–violet-brown	–Cysteine	– bluish-green
Glycyl-leucine	–violet-brown	–Glycine	– bluish-green
Glycyl-tyrosine	–violet-brown	–Leucine	– bluish-green
Glycyl-tryptophan	–violet-brown	–Alanine	– bluish-green
Leucyl-glycine	–green-yellow	–Tyrosine	– grey-brown
Alanyl-glycine	–green-yellow	–Tryptophan	– grey-brown
Cysteinyl-glycine (+ glutamic acid)	–pink-yellow		

Instead of cysteinyl-glycine, a mixture of cysteinyl-glycine and glutamic acid (partial acidic hydrolysate of glutathion[4]) was used. All substances were Hoffmann-La Roche products.

[1] G. SEMENZA, Biochim. biophys. Acta 1957 (in press).
[2] F. BINKLEY, Exp. Cell. Res. Suppl. *2*, 145 (1952).
[3] K. V. GIRI and A. NAGABHUSHANAN, Naturwissenschaften *39*, 548 (1952).

As seen from the above table, a differentiation is possible between several dipeptides and their respective component amino acides.

Test for the cysteinyl-glycinase activity. The following procedure was used either to detect the cysteinyl-glycinase on the electrophoresis paper, or as a spot test of a solution to be analyzed.

The filter paper carrying a drop of the solution, or the electrophoresis paper, was dipped in a uniform thin layer of the substrate-buffer-activator mixture: neutralized hydrolyzed glutathion[4] 3 to 4 mg per millilitre, corresponding to about 1·5 to 2 mg cysteinyl-glycine per millilitre, in 0·02 *M* THAM-HCl[5] buffer, pH 8·1 + 0·0005 *M* $MnCl_2$.

Paper electrophoresis of cysteinyl-glycinase. 0·04 *M* THAM-HCl buffer, pH 8·2, + 0,0005 *M* $MnCl_2$; 400 V during 3 h at room temperature on Whatman N 54. The cysteinyl-glycinase activity is shown by the green-grey spot (white in the figure) against a pink background (black in the figure).

Incubation was carried out at room temperature for 30–45 min, and stopped by heating the paper in the oven at 110°C for 5 min. The paper was then stained according to the procedure described above; the cysteinyl-glycinase activity was shown by the appearence of a violet (after the heating) or green-bluish (in the alcohol-alkali bath) spot, against a pink-yellow background.

Alternatively, the incubation was carried out by superposing the paper into a 1% agar gel, containing the substrate-buffer-activator mixture. The agar gel was then stained by spraying the reagent *a*: the colour developed slowly at room temperature, without any further treatment. The cysteinyl-glycinase activity was shown by the appearence of a brown spot, against a yellow background. The electrophoresis paper, in this alternative procedure, could of course be used for other analysis.

The sensitivity of these procedures is somewhat higher than the usual quantitative method in a tube[4].

To test other dipeptidase activities, an identical procedure was used. The reaction mixture contained about 3 mg of dipeptide per millilitre, the buffer and the suitable activator.

G. SEMENZA*

Institute of Biochemistry, University of Uppsala, Sweden, December 19, 1956.

Riassunto

Viene descritto un metodo rapido e sensibile per la rilevazione delle dipeptidasi su carta.

[4] F. BINKLEY, J. biol. Chem. *186*, 731 (1950).
[5] Tris-hydroxymethyl-amino-methane Sigma.
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