Effect of Iproniazid on Monoamines and Monoamine Oxidase in Human Brain

Iproniazid is known to be a potent inhibitor of monoamine oxidase (MAO) both *in vitro* and *in vivo* according to animal experiments[1–4]. By following urinary excretion of monoamines as well as of their end products, it has been demonstrated that Iproniazid inhibits MAO also in man[5–7]. Such studies give, however, no information on the effect on brain MAO, which may differ considerably from that of other organs.

Iproniazid and other MAO inhibitors have been much used in the treatment of patients with depressions. The drugs have also been given to patients with angina pectoris and far-advanced cancer, in which cases they have been reported to be useful for the management of the severe pain[8–10].

In connection with clinical trials where patients with far-advanced cancer were treated with MAO inhibitors, it was possible to perform the investigation presented in this paper. We have found it of use to make this examination, as we hereby have a certain possibility in humans to confirm results, obtained in animal experiments. Also it is of value for the clinicians to get information on the effect of MAO inhibitors in human brain, when therapeutic doses are used.

The material consisted of 5 patients who had received Iproniazid and of a control series of 11 patients. In the Iproniazid treated group, all of the patients received Iproniazid on the indication of metastatic pain. In the control series, most of the patients had died of cancer or circulatory disorders. They were selected in the autopsyroom and some of them had been ill for a long time and were emaciated at the time of death, while others had died suddenly after a short illness. In both groups the patients were 40–60 years of age.

The body was sent to the mortuary within 2 h of death and was autopsied within 3–70 h. Bodies not autopsied immediately were kept in a room at + 4°C. At the autopsy, we took from the brain pieces of the cortex from the parietal region, the head of the caudate nucleus on one side, the mesencephalon, and one piece from the liver. The pieces were stored at – 20°C until examined for MAO activity. The caudate nucleus on the other side and the hypothalamus were excised and put into 10 ml of 0.4 *N* perchloric acid before they were frozen. These specimens were used for examination of monoamines. The determinations were made without undue delay, usually on the same day.

The MAO activity was measured manometrically by incubation in an athmosphere of oxygen at 37°C. Homogenized tissue was incubated with tyramine, potassium cyanide and semicarbazide according to CREASEY[11] with the modification that 0.1 *M* Tris buffer of pH 8.0 was used. The homogenization of the tissues was performed in an 'Ultra-Turrax' homogenizer together with the Tris buffer. Tissue specimens treated and incubated in the same way but without tyramine were used as blanks. The difference between the oxygen consumption by the tissue incubated with and without tyramine was taken as a measure of the MAO activity. The noradrenalin (NA) and the 5-hydroxytryptamine (5-HT) contents in the hypothalamus were determined by the methods of BERTLER, CARLSSON, and ROSENGREN[12] and BERTLER and ROSENGREN[13] respectively. The dopamine (DA) content of the caudate nucleus was measured according to CARLSSON and WALDECK[14].

MAO activity was found to be very resistent to postmortal destruction (Figure 1). Thus in the controls no appreciable difference was found between the values obtained from patients not autopsied until 70 h after death and values from those autopsied earlier. The mean MAO activity found in the cortex in the 11 controls was 9 μmoles/g/h (range 7–12 μmoles/g/h). The activity found in the caudate nucleus as well as in the mesencephalon was about 16 μmoles/g/h with a moderate range of variation. A somewhat greater variation was observed in the liver MAO activity, 50–100 μmoles/g/h (mean 68 μmoles/g/h). The lowest values were recorded from livers with fibrosis. Since the range of variation of the values in the controls was only moderate, it may be assumed that the diseases from which the patients had died had not sub-

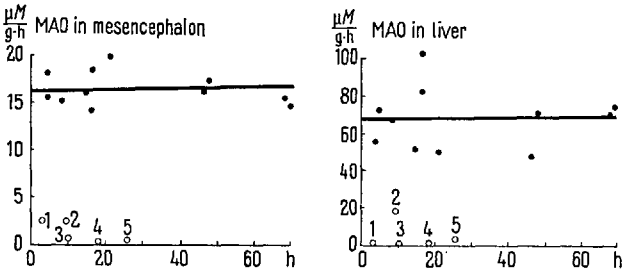

Fig. 1. Monoamine oxidase activity in μmoles/g/h in the mesencephalon and the liver plotted against interval between death and autopsy. Circles designate the 5 cases treated with Iproniazid; dots the controls. The regression of activity upon time has been calculated for the control group by the method of least squares.

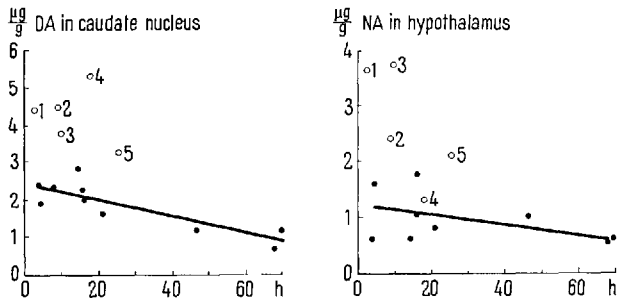

Fig. 2. Concentration in μg/g of dopamine in the caudate nucleus and of noradrenalin in the hypothamalus plotted against interval between death and autopsy. Circles designate the 5 cases treated with Iproniazid; dots the controls. The regression of concentration upon time has been calculated for the control group by the method of least squares.

[1] E. A. ZELLER, J. BARSKY, E. R. BERMAN, and J. R. FOUTS, J. lab. clin. Med. *40*, 965 (1952).
[2] E. A. ZELLER, J. BARSKY, and E. R. BERMAN, J. biol. Chem. *214*, 267 (1955).
[3] K. F. GEY and A. PLETSCHER, J. Neurochem. *6*, 239 (1961).
[4] H. WEISSBACH, W. LOVENBERG, B. G. REDFIELD, and S. UDENFRIEND, J. Pharmacol. exp. Therap. *131*, 26 (1961).
[5] A. SJOERDSMA, L. GILLESPIE, and S. UDENFRIEND, Ann. N.Y. Acad. Sci. *80*, 969 (1959).
[6] J. A. OATES and P. ZALTMAN, Ann. N.Y. Acad. Sci. *80*, 977 (1959).
[7] W. v. STUDNITZ, Scand. J. clin. lab. Invest. *11*, 101 (1959).
[8] J. BATEMAN, J. clin. exp. Psychopath. *19*, Suppl. 1, 123 (1958).
[9] J. V. PRICE, J. Soc. Ciencias med. Lisboa *123*, Suppl. 337 (1959).
[10] D. M. BOSWORTH, Ann. N.Y. Acad. Sci. *80*, 809 (1959).
[11] N. H. CREASEY, Biochem. J. *64*, 178 (1956).
[12] Å. BERTLER, A. CARLSSON, and E. ROSENGREN, Acta physiol. Scand. *44*, 293 (1958).
[13] Å. BERTLER and E. ROSENGREN, Exper. *15*, 382 (1959).
[14] A. CARLSSON and B. WALDECK, Acta physiol. Scand. *44*, 293 (1958).

stantially influenced the MAO activity. No significant difference could be demonstrated between the values obtained from patients who had died after a long period of illness and from those who had died suddenly.

In the 5 cases treated with Iproniazid the values found were much lower than those observed in the controls (Figure 1). Thus in 3 cases (Nos. 3, 4, 5) treated 1–3 weeks before death with 75–125 mg daily, the MAO activity was less than 5% of the mean control value. In one case (No. 1), that had been treated with Iproniazid only on the last 4 days before death with a dose of 50 mg daily intramuscularly, the activity in the parts of the brain studied was 15% of the mean control values. The inhibition of the liver MAO in this case was still more pronounced despite the smallness of the dose of Iproniazid, the activity being less than 3% of the mean control value. This indicates that there may be a difference between the degree of MAO inhibition in different organs after treatment with MAO inhibitors. The present experiments indicate that the MAO of the liver is more accessible to Iproniazid than that of the brain. Similar observations have been made in animals[15]. It should be observed that in the case mentioned above the patient had received the Iproniazid parenterally. In another case (No. 2), which had been given Iproniazid in a dose of 50–100 mg daily for 4 weeks until 6 days before death, the activity in the brain was 15% of that in the controls, the corresponding value in the liver being about 25%. In this case, then, the inhibition was more marked in the brain, which might indicate a less rapid turnover of the brain MAO. The results from a more recent case, not included in the Figures, were in agreement with the latter observation.

The range of variation of the monoamines in the brain was somewhat wider than that of the MAO activity (Figure 2). A slow decrease of the concentration of catecholamines during the interval between death and necropsy was noted. The values observed for the DA and NA concentrations will appear from Figure 2. The mean value found for the 5-HT concentration in the hypothalamus was 0.16 μg/g. This mean was obtained from cases autopsied 10–15 h after death. The number of 5-HT determinations in the control group was not enough to assess the rate of fall, if any, between death and necropsy.

Administration of Iproniazid caused a higher monoamine level in the brain. The mean concentration of DA in the caudate nucleus and of NA and 5-HT in the hypothalamus was about twice as high in the cases which had received Iproniazid as in the controls (Figure 2). There appears to be no reason to believe that the monoamine levels observed did not reflect the concentrations present before death. The assumption is strengthened by earlier observations made in some cases that the amount of 3,4-dihydroxyphenylacetic acid, the end product after oxidative deamination of DA, was low in human brain. This indicates that no appreciable breakdown of DA by means of MAO takes place after death. It is also in agreement with the results from similar experiments made on rats in which the determinations could be made immediately after death[3]. The increase of catecholamines and of 5-HT after inhibition of MAO therefore seems to indicate that this enzyme is of importance for the metabolism of both catecholamines and 5-HT in the human brain.

The observations made in the present investigation suggest that treatment with Iproniazid in ordinary therapeutic doses has an inhibitory effect on the MAO in the brain and in the liver, and that it increases the concentration of monoamines. The psychostimulating effect of monoamine oxidase inhibitors might therefore be due to an increase of brain monoamine levels[16].

Zusammenfassung. Iproniazid in therapeutischen Dosen verursacht beim Menschen eine ausgesprochene Hemmung der Gehirn-Monoaminoxidase und eine Verdoppelung des Gehalts an 3-Hydroxytyramin, Nor-adrenalin und 5-Hydroxytryptamin.

P. O. Ganrot, E. Rosengren, and C. G. Gottfries

Department of Pharmacology, University of Lund (Sweden), and Östra Sjukhuset, Malmö (Mental Hospital of Malmö, Sweden), January 24, 1962.

[15] A. N. Davison, A. W. Lessin, and M. W. Parkes, Exper. *13*, 329 (1957).

[16] For his kind cooperation we are greatly indepted to M. D. A. Grönblad, the head of the Hospital for Chronic Diseases, Malmö, where the present therapeutic trials were performed. This work has been supported by grants from the Swedish Medical Research Council.

Conversion of β-Mercaptopyruvate to 2-Mercaptoethanol by Yeast Enzymes[1]

β-Mercaptopyruvic acid arises in animal tissues by the transamination of cysteine with an α-keto acid[2]. The metabolic fate of β-mercaptopyruvate in animal tissues has been investigated extensively. The mechanism of enzymic removal of sulfur, a reaction first observed by Meister[2], has been studied in detail in our laboratory[3–7]. Although this reaction probably is the most important pathway for enzymic degradation of β-mercaptopyruvate in animal tissues, its reduction by dihydro diphosphopyridine nucleotide (DPNH) and lactic dehydrogenase to mercaptolactic acid[8,9] is sufficiently rapid to be of physiological importance. However, certain cells (e.g. yeast) do not contain appreciable amounts of the sulfur removing enzyme and pyridine nucleotide-linked lactate dehydrogenase. Alternate pathways for the degradation of β-mercapropyruvate may therefore assume greater significance. It was indeed observed that yeast extracts produce CO_2 when incubated with β-mercaptopyruvate[8].

We have pursued the problem of the enzymatic decarboxylation of β-mercaptopyruvate in order to establish the reaction sequence in a yeast enzyme system, capable of metabolizing this acid to 2-mercaptoethanol.

[1] Supported by grants of the USPH, C-4681 and C-3211, and by a grant of the American Heart Association Inc., New York.

[2] A. Meister, P. E. Fraser, and S. V. Tice, J. biol. Chem. *206*, 561 (1954).

[3] E. Kun and D. W. Fanshier, Biochim. biophys. Acta *27*, 658 (1958).

[4] E. Kun and D. W. Fanshier, Biochim. biophys. Acta *33*, 28 (1959).

[5] E. Kun and D. W. Fanshier, Biochim. biophys. Acta *32*, 338 (1959).

[6] E. Kun and D. W. Fanshier, Biochim. biophys. Acta *48*, 187 (1961).

[7] D. W. Fanshier and E. Kun, Biochim. biophys. Acta, in press (1962).

[8] A. Meister, J. biol. Chem. *197*, 309 (1952).

[9] E. Kun, Biochim. biophys. Acta *25*, 135 (1957).