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3 J.W. Everett, C.H. Sawyer and J.E. Markee, Endocrinology *44*, 234 (1949).
4 J.W. Everett and C.H. Sawyer, Endocrinology *47*, 198 (1950).
5 L.C. Krey and J.W. Everett, Endocrinology *93*, 377 (1973).
6 J. Roos, M. Roos and Cl. Aron, Bull. Ass. Anat., Paris *59*, 499 (1975).
7 M. Hassani and S. Plas-Roser, Archs Biol., Liège *86*, 435 (1975).
8 L.C. Krey, L. Tirey and J.W. Everett, Endocrinology *93*, 385 (1973).
9 S. Plas-Roser, M. Hassani and Cl. Aron, Acta endocr., Copenh. *85*, 151 (1977).
10 K. Uchida, M. Kadowaki, T. Miyake and K. Wakabayashi, Endocr. jap. *20*, 103 (1973).
11 B. Kerdelhue, S. Pitoulis and A. Berault, in: Symposium sur les techniques radioimmunologiques, p.257. INSERM publ. Paris 1972.
12 One female in this group did not respond to EB.
13 S.J. Legan, G.A. Coon and F.J. Karsch, Endocrinology *96*, 50 (1975).
14 C.A. Blake, Endocrinology *101*, 1122 (1977).
15 G. Chazal, M. Faudon, F. Gogan, M. Hery, C. Kordon and E. Laplante, J. Endocr. *75*, 251 (1977).
16 S.J. Legan and F.J. Karsch, Endocrinology *96*, 57 (1975).

Binding of iproniazid to the polymeric forms of iodide peroxidase

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Summary. ^{14}C labelled iproniazid binds to iodide peroxidase more effectively in low ionic strength buffer than in high ionic strength buffer, suggesting preferential binding to the monomeric form of iodide peroxidase. During column chromatography, under conditions that separate iodide peroxidase into multiple forms, iproniazid is bound selectively to the monomeric form. Thus, this antithyroid agent appears to bind preferentially to the monomeric enzyme form, or possibly to cause dissociation of the polymeric to the monomeric form.

Although there are many inhibitors of thyroid peroxidase, the details of their inhibitory mechanisms have not been elucidated.We have investigated the action of iproniazid, an inactivator of iodide peroxidase, in relation to the structure of the enzyme.

Iproniazid(isonicotinyl isopropyl hydrazine), an irreversible inhibitor of monoamine oxidase, was observed to inhibit horse radish peroxidase (HRP)[2]. Iproniazid binds to the nonheme portion of HRP irreversibly and stoichiometrically[2]. As with HRP, iproniazid binds to purified iodide peroxidase and inhibits the enzyme[3]. Spectral changes produced by iproniazid in iodide peroxidase were similar to those produced with HRP[4]. The binding is believed to be covalent.

Recently, iodide peroxidase was reported by Nagasaka and DeGroot to exist in polymeric forms[5]. We, in this communication, demonstrate differences in the sensitivity of these polymeric forms of enzyme to iproniazid.

Methods. Calf thyroids were obtained from an abattoir and kept on ice until dissected. Solubilization and purification of the iodide peroxidase enzyme were described in a previous paper[6]. Polymeric forms of iodide peroxidase were prepared using sephadex G-200 chromatography. Details of the method of preparation were described in a previous paper[5].

Enzyme activity was determined by measuring ^{131}I incorporation into tyrosine. Details of the assay were reported previously[6]. All chemicals were obtained from commercial sources unless otherwise stated.

^{14}C iproniazid was prepared by Dr H. Kaegi and Dr W. Burger at Hoffmann-La Roche and 1–5 μCi was used after dilution with unlabelled iproniazid. Preincubations of the enzyme with iproniazid ($1\text{–}3 \times 10^{-3}$ M) were carried out at 37 °C for 2 h in varying concentrations of phosphate buffer (from 0.005 M to 0.2 M, pH 7.0). Following incubation of the enzyme with iproniazid (in 0.5 ml) for the periods of time shown in the legends, 0.1 ml of this solution was diluted 5-fold to measure the residual enzyme activity and 10% TCA was added to the residual reaction mixture to stop the reaction and the solution was dialyzed against water (1000 vol.) overnight. After dialysis, precipitated protein was collected, dissolved in NCS (Packard) solution, and the radioactivity bound to the protein was measured in Bray's solution by liquid scintillation counting.

To prepare iproniazid-bound iodide peroxidase for column chromatography, solubilized iodinating enzyme[6] was precipitated by 40% saturated ammonium sulfate solution,

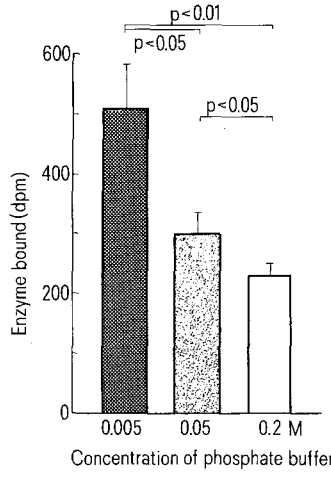

Fig. 1. Effect of ionic strength on iproniazid binding to iodide peroxidase. The ^{14}C radioactivity of iproniazid (1 μCi ^{14}C iproniazid and 1 μmole stable iproniazid per ml) bound to iodide peroxidase after 1 h incubation in 0.005 M (▩), 0.05 M (▨), and 0.2 M (□), pH 7.0, phosphate buffer is graphed. Each bar represents mean ± SE of 5 experiments. Student's t-test was applied for statistical analysis.

filtered on an Amicon PM-10 filter to remove ammonium sulfate, and made up in 0.005 M phosphate buffer, pH 7.0, with 10^{-5} M KI and 0.01% Triton × 100. The enzyme was incubated with iproniazid (3×10^{-3} M iproniazid and 1–5 μCi ^{14}C-iproniazid) at 37 °C for 2 h. After incubation, the reaction mixture was allowed to stand at room temperature overnight, and then dialyzed against water (1000 vol.) for 24 h and applied to a sephadex G-200 column (2.5 × 75 cm).

As reported previously[5], iodide peroxidase activity appears partially to dissociate from a polymer to a monomer on chromatography through sephadex G-200 in low ionic strength buffer (0.005 M, pH 7.0). This phenomenon is prevented by higher ionic strength phosphate buffer. Binding of iproniazid to enzyme was examined after incubation in 0.005 M phosphate buffer.

Results. As shown in figure 1, at concentrations over 0.05 M of phosphate buffer, the amount of iproniazid irreversibly bound to enzyme was lower than obtained at concentrations below 0.05 M. These facts suggest that the tetramer is less sensitive to iproniazid-binding than the monomer, the form favored at low concentrations of phosphate buffer[5].

Iodide peroxidase-tyrosine iodinase tetramer was purified by sephadex G-200 chromatography. More ^{14}C-iproniazid was bound to the purified polymer in diluted sodium phosphate buffer (0.005 M), a condition favoring dissociation to the monomeric form, than when enzyme was incubated in 0.2 M buffer. Iproniazid inhibition of enzymatic iodination was also greater when enzyme was incubated with the iproniazid in dilute buffer (0.005 M buffer) (figure 2).

The elution profile of iproniazid-labelled iodide peroxidase was compared to that of untreated enzyme during sephadex G-200 chromatography. The labelled enzyme solution was dialyzed against 1000 vol. of 0.005 M phosphate buffer, pH 7.0, before application to the column. As shown in figure 3 A, in a control run, 3 peaks (Nos 1, 2 and 3) of iodide peroxidase which have different molecular sizes[5] were observed. After iproniazid treatment, the radioactivity was found to associate with peak No. 1 (in which iproniazid bound to nonenzymatic protein would be included) and peak No. 3, the monomeric form (figure 3, B). After filtration of the radioactive peaks No. 1, and No. 3 on an Amicon filter PM-10 (molecules over 10,000 mol.wt should be retained), radioactivity of these peaks was retained by the filter, indicating that the radioactivity of iproniazid remained bound to the enzyme. In this experiment, no radioactive iproniazid was found in peak No. 2. This implies 2 possibilities, that iproniazid may more readily bind to the monomer than to the tetramer, or that binding causes dissociation to the monomer.

Discussion. Iproniazid binds to the monomeric form of iodide peroxidase, and less or not at all to the polymeric form. This phenomenon is demonstrable by experiments in which the formation of monomer is augmented by low ionic strength buffer.

The evidence is conclusive in experiments involving sephadex chromatography of the enzyme, for under these conditions separation of the 2 forms of enzyme is assured.

The reason why the tetramer interacts with iproniazid less than the monomer is not clear, but it might be possible that a conformational change produced by polymerization prevents binding and inactivation by iproniazid. Another possibility is that iproniazid favors dissociation of enzyme to the unstable monomeric form.

Further studies with other antithyroid drugs one of which inhibits thyroidal iodide peroxidase irreversibly[7], both in vitro and in vivo, are planned, to evaluate whether the action of iproniazid is unique, or possibly typical of 1 class of antithyroid agents.

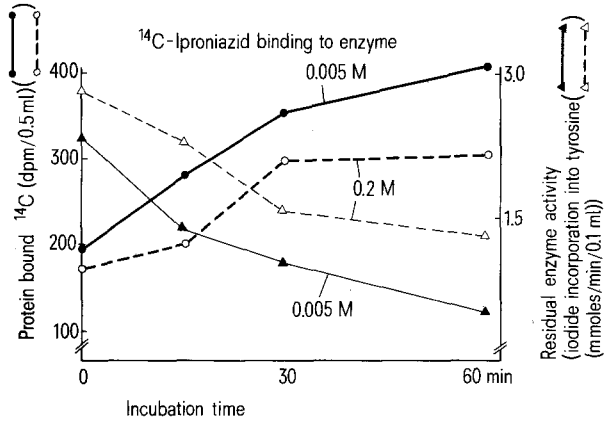

Fig. 2. Iodide peroxidase-tyrosine iodinase tetramer (4.75 mg protein) was incubated with 3×10^{-3} M iproniazid and 5 μCi ^{14}C-iproniazid in 4 ml of 0.005 M or 0.2 M sodium phosphate, pH 7.0, buffer at 37 °C for 0–60 min. Aliquots were removed at intervals for determination of ^{14}C radioactivity bound to protein (0.5 ml of aliquots), and residual peroxidase activity (0.1 ml of aliquots), assayed as described above. The enzyme incubated with iproniazid revealed lower activity than that of the control and same ^{14}C binding at the zero time in the figure because iproniazid binding to the enzyme occurred during the 20 min incubation for peroxidase assay. Each value shown is the mean of duplicate experiments.

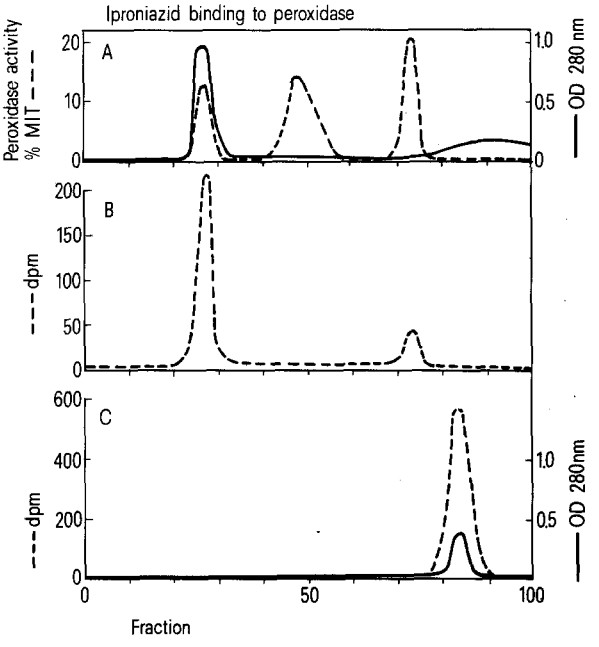

Fig. 3. Iproniazid binding to peroxidase. Elution profile of iodide peroxidase and ^{14}C-iproniazid during sephadex G-200 chromatography. *A* Control run in 0.005 M sodium phosphate buffer including 10^{-5} M KI and 0.01% triton. The peaks of enzyme activity are designated peak Nos 1, 2, and 3 in order, from the left side. *B* Iproniazid-labelled iodide peroxidase in the same buffer. *C* ^{14}C-iproniazid and stable iproniazid, without enzyme, in the same buffer. The OD peak at fractions 80–90 is due to iproniazid. % MIT (% monoiodotyrosine) indicates percent incorporation of iodide into tyrosine by peroxidase. The protein, shown by absorbance at 280 nm (OD_{280} nm), was eluted mainly in peak No. 1 of iodide peroxidase (this protein peak may be contaminated with nonenzymatic and large molecular protein). Small amounts of the protein were eluted at peaks Nos 2 and 3 of iodide peroxidase. This indicates there was little contamination by nonenzymatic protein in peaks Nos 2 and 3 of the enzyme.

1 Supported in part by United States Public Health Service Grants AM-13,377 and AM-13,643.
2 H. Hidaka and S. Udenfriend, Archs Bioch. Biophys. *140,* 174 (1970).
3 H. Hidaka, A. Nagasaka and L.J. DeGroot, Endocrinology *88,* 1264 (1971).
4 H. Hidaka, S. Udenfriend, A. Nagasaka and L.J. DeGroot, Biochem. biophys. Res. Commun *40,* 103 (1970).
5 A. Nagasaka and L.J. DeGroot, Endocrinology *88,* 470 (1971).
6 L.J. DeGroot and A.M. Davis, Endocrinology *70,* 492 (1962).
7 A. Nagasaka and H. Hidaka, J. clin. Endocr. Metab. *43,* 152 (1976).

Effect of repeated electroconvulsive shock on plasma noradrenaline and adrenaline in man

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Summary. The concentration of noradrenaline (NA) and of adrenaline (A) in plasma was measured before and 3, 30 and 60 min after single and repeated electroconvulsive shocks (ECS). Single ECS resulted in an activation of the sympatho-adrenal medullary system; however, after the treatment had been repeated 4 times there was evidence of a diminished response of the peripheral sympathetic nervous system in comparison to the response to the first ECS.

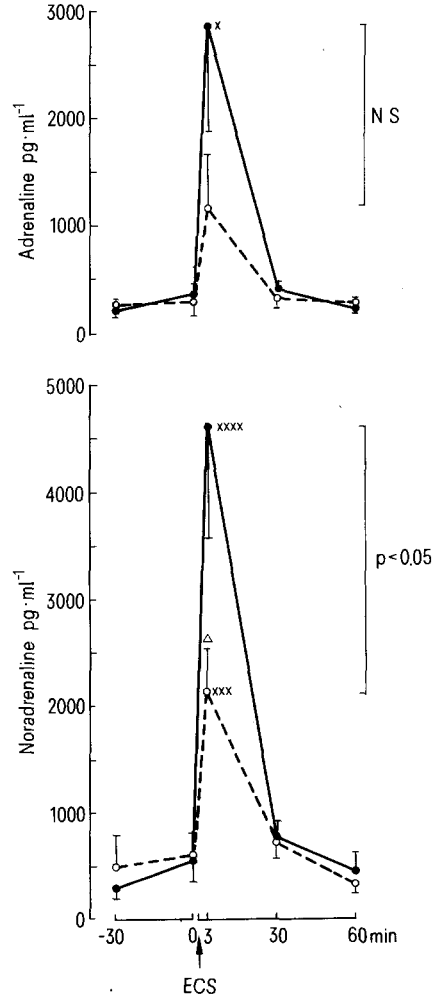
Electroconvulsive shock has been shown to affect several biochemical parameters of the central noradrenergic system in the rat. It increases the activity of tyrosine hydroxylase[1] and the turnover and release of NA[2], and decreases the high affinity uptake of NA into synaptosomes[3]. Moreover, the sympatho-adrenal medullary activity of rats exposed to footshock was also found to be increased[4].

In the present study, we have examined the sympathoadrenal medullary response of man (psychiatric patients) to single and repeated ECS.

Materials and methods. The experiments were performed at the Regional Mental Hospital in Pezinok, with psychiatric patients for whom ECS without anticonvulsive premedication (ACP) was indicated for psychiatric reasons as a routine procedure. The study included 5 males, ranging in age from 26 to 52 years, with body weights within the normal range. 4 patients were diagnosed as schizophrenic and 1 as endogenously depressive. All patients were receiving antipsychotic drugs (chlorpromazin, chlorprothixen or clozapin) which had been omitted only on the day of ECS. During the last 6 months before our investigation the patients had not been subjected to ECS. After overnight fasting ECS was applied between 7.30 and 8.30 h by bitemporal electrodes (400–500 mA at 120 V during 0.9–1.1 sec). Special care was taken to prevent injury during convulsions. Venous blood samples were taken 30 and 0 min before ECS and 3–5, 30 and 60 min after the ECS, both during the first and the 4th ECS. In all cases the 4 ECS were applied during a 8-day period. A modification of a sensitive radioenzymatic assay was used to measure plasma NA and A[5,6]. Student's t-test for unpaired values and analysis of variance were used to determine the statistical significance. Results are given as means ± SEM.

Results and discussion. Values for NA and A are depicted in the figure. Immediately after the first ECS, NA peaked up to a level of about 4600 pg/ml. The 4th repetition of ECS was, however, connected with a significant diminution ($p < 0.05$) of the NA response which still was increased significantly ($p < 0.01$). On the other hand, although the plasma A response to the 4th ECS was not statistically significant compared to the preshock level, no significant difference between plasma A levels during the 1st and 4th ECS could be registered. All statistical calculations made by Student's t-test were confirmed by the analysis of variance.

Exposure of psychiatric patients to an intensive stress stimulus (i.e. ECS without ACP) resulted in an activation of the sympatho-adrenal medullary system as evidenced by striking increments in circulating levels of NA and A. A similar finding has been described earlier in the rat[4]. However, it should be mentioned that no activation of the sympathetic nervous system after ECS with ACP was



Pattern of plasma NA and A response to the 1st and the 4th electroconvulsive shock in 5 psychiatric patients. Statistical significances to preshock values are indicated by asterisks. x, xxx and xxxx belong to $p < 0.05$, 0.01 and 0.002, respectively. $\Delta = p < 0.05$ compared to 1st exposure.