ACTION OF POTASSIUM IODIDE ON THYROID ACID PROTEASE

By
M. A. Pisarev and N. Altschuler

ABSTRACT

Potassium iodide (KI) is known to inhibit thyroid hormone secretion. In the present studies its action on the proteolytic step of this process was investigated. Rats were treated with KI (200 μg/ml in the drinking water) for 30 days. This treatment caused a decrease of protease activity in total homogenate and in the specific activity of a 15,000 × g pellet. No alteration in the pattern of subcellular distribution was observed. In order to rule out an action of KI on enzyme activity its in vitro action was studied. KI concentrations around 10⁻³–10⁻⁴ M were without effect, though 10⁻² caused a stimulation of activity. Similar results were observed when a liver enzyme preparation was checked under the same conditions. Neither Cl⁻ nor F⁻ had an effect on thyroid or liver protease at concentrations between 10⁻² to 10⁻⁴ M. The present results suggest that KI inhibition of thyroid hormone secretion can be explained at least in part by its action on acid protease. Moreover, the lack of an in vitro inhibitory affect of KI would suggest that this drug affects enzyme synthesis and/or breakdown.

Potassium iodide (KI) has been used for many years in the amelioration of Graves’ disease symptoms and in the treatment of diffuse euthyroid goitre.

* Established investigators of the Conicet.
Supported in part by a grant from the Consejo Nacional de Investigaciones Científicas y Técnicas (Conicet), Argentina.
This work was carried out under Contract of the Ministère de la Politique Scientifique.

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Solomon (1956) and Greer & DeGroot (1956) have shown that it decreases hormone release in humans. In recent years the action of KI was studied in different experiments. Thus, KI has been shown to block the production of goitre by thyrotrophin (TSH), cyclic AMP and cyclic GMP (Katakai et al. 1966; Pisarev & Itoiz 1972) as well as stimulation of thyroid hormone secretion by TSH, LATS and cyclic AMP (Ochi & DeGroot 1969; Pisarev et al. 1971). All these studies suggested that KI exerted its effect at a step distal to the cyclic nucleotide formation. However it is impossible from all the data available to indicate the “locus” of the KI action (for a detailed review see Wolff (1969)).

The thyroid hormone secretion process include several steps, namely, endocytosis of thyroglobulin, fusion of colloid droplets with lysosomes digestion of thyroglobulin by an acid protease, dehalogenation of iodothyrosines and release of iodothyronones into the blood stream. In order to get further insight into the mechanism of action the effects of this drug on thyroid acid protease were investigated. The present results demonstrate that chronic administration of KI to rats decrease enzyme activity in thyroid tissue and that its effect may be mediated through an action on protease synthesis and/or breakdown.

**MATERIALS AND METHODS**

Wistar rats 150–180 g body weight were used. The animals were fed with Forramez pellets (Molinos Rio de la Plata, Argentina) and water ad libitum. Groups of rats were treated during 30 days with KI, 200 μg/ml in the drinking water. The animals were sacrificed by a blow in the neck and the thyroid gland was carefully dissected, weighed and homogenized in pools of 4 rats each. Homogenization was performed in a Potter Elvehjem type (all glass) tissue homogenator in 0.25 m cold sucrose solution. The homogenate was centrifuged in a Sorvall RC-2 refrigerated centrifuge at 800×g (20 min). The pellets obtained after each centrifugation were washed twice with the sucrose solution and the supernatants were combined. Three subcellular fractions were obtained: N (precipitate of 800×g) L–M (precipitate of 15,000×g) and the supernatant (S). Fractions N and L were resuspended in 1 ml of cold distilled water, and after several minutes on ice were spun at 800×g and 15,000×g, respectively, as described above. The supernatants were used for enzyme assay and protein estimation.

Acid protease activity was determined using bovine haemoglobin (Hb) as substrate. The incubation mixture contained 0.2 ml of tissue preparation, 0.5 ml of citric acid: Na citrate buffer pH 3.5 and 0.5 ml of a 2% Hb solution in the same buffer. The haemoglobin had previously been filtered. Incubations were performed for different periods of time at 37°C in a Dubnoff equipment with shaking and stopped by the addition of 0.5 ml of 20% cold trichloroacetic acid (TCA). The samples were centrifuged at 3000 r. p. m. during 30 min and the 280 nm absorbance of the supernatant determined. Each set of tubes had its blank obtained from tubes in which TCA was added at the same time as the enzyme preparations. All the samples were run in duplicate.

In other series of experiments the action of different substances on acid protease
activity was studied. Enzyme preparations were obtained from the thyroid and from the liver as described above.

Recovery of total enzyme activity was calculated after differential centrifugation as compared to total homogenate and accounted for 90 to 100% of the total activity. Protein was determined according to Warburg & Christian (1941). Subcellular distribution and specific activity of the enzymatic activity were calculated. Statistical analysis was done according to Student’s t-test.

RESULTS

No change was observed in thyroid gland weight after 30 days of KI treatment. Fig. 1 shows protease activity during different periods of time. A linear relationship between different amounts of preparation and enzyme activity was obtained as observed in Fig. 2. Control studies were performed in order to establish the reason for the relatively high blank values (“0” enzyme). Aliquots of Hb were incubated in duplicate for different periods of time (“0”, 30 min, 3 h and 24 h). All samples had the same amount of material absorbing at 280 nm (e.g.: 0.075, equal to our 75 units). These results indicate that this Hb preparation contains TCA soluble material, thus explaining our blank values. Moreover, incubation without enzyme failed to cause any further increase in the absorbance.

Fig. 1.
Curve of protease activity during different periods of incubation. Units are equal to the difference of optical density between experimental tubes and their blanks in relation to 0.2 ml of enzyme preparation. One unit = 0.001 OD_280. Each value is an average of duplicates.
The pH optimum, studied for both enzymatic activities present in the 15,000 x g pellet (resuspended in citric acid buffer) and in the supernatant, was around 3.5 (Fig. 3).

KI caused a statistically significant decrease of protease activity in total homogenate (Table 1). The highest specific activity of the enzyme was found in the LM fraction and this latter was significantly decreased after in vivo KI treatment (Table 1). The pattern of subcellular distribution was not changed in these animals. Sedimentable and soluble enzyme values were 42.0, 48.8, 57.0 and 51.2% of total activity for the control and KI treatment rats, respectively.

In order to rule out the possibility that KI was acting directly on the enzyme activity the following in vitro experiments were performed. The action of different KI concentrations on acid protease was studied. 10^{-3} to 10^{-4} M KI
Fig. 3.

pH optimum curve for protease preparations obtained from the 15,000 x g pellet or from its supernatant.

did not change the enzyme activity after 2 h incubations; but a relatively high concentration (10^{-2} M) increased the enzyme action in both preparations studied (Fig. 4). In other series of experiments the action of other halogens (Cl^- and F^-) was investigated. None of them caused a change in thyroid acid protease

Table 1.

Action of in vivo KI administration of thyroid acid protease.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>KI-treated</th>
<th>P &lt;</th>
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</thead>
<tbody>
<tr>
<td><strong>Protease activity</strong></td>
<td></td>
<td></td>
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<tr>
<td>Total homogenate U/mg gland</td>
<td>367.6 ± 16.4</td>
<td>211.7 ± 47.0</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Distribution of enzyme specific activity</strong></td>
<td>(units/mg prot.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>800 x g pellet</td>
<td>114.2 ± 10.7</td>
<td>100.0 ± 12.5</td>
<td>n.s.</td>
</tr>
<tr>
<td>15 000 x g pellet</td>
<td>264.3 ± 50.0</td>
<td>133.9 ± 14.3</td>
<td>0.005</td>
</tr>
<tr>
<td>Supernatant</td>
<td>132.1 ± 10.7</td>
<td>121.4 ± 17.8</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Protease activity is expressed in terms of optical density arbitrary units (1 U = 0.001 OD_{280} nm). Each value is an average of four determinations ± 1 sd.
activity. Similar results were obtained with rat liver acid protease. KI concentrations of 10^{-2} \text{ M} caused a significant increase in enzyme activity, while lower concentration of this salt and NaCl or NaF (10^{-2} \text{ to } 10^{-4} \text{ M}) failed to alter the basal values.

**DISCUSSION**

Acid protease activity was found in both LM and S fractions. The highest specific activity was observed in the former, in agreement with previous results (Herveg et al. 1966) which suggested the lysosomal localization of this enzyme. It is likely that part, if not all, of the enzyme appearing in the soluble fraction was released from the lysosomes during manipulation, but similar results were obtained with other procedures (Jablonski & McQuillan 1967). Thus the question as to whether there is a true localization of the enzyme in the cell sap
remains open until better procedures for cell distribution become available (see Wollman (1969) for discussion of this topic). The similar pattern observed for both enzyme (S and LM) preparations, as far as pH optimum is concerned is compatible with a common nature (or origin) for both.

The present result demonstrates that chronic KI administration to rats causes a decrease of acid protease activity in total homogenate as well as in the LM fraction specific activity. Takeuchi et al. (1970) also found that chronic KI treatment depressed autoproteolytic activity in the rat thyroid and Dziemian (1943) reported, using a micro-titration procedure, that KI feeding to rats caused a 15–30% increase in proteolytic activity between the first and second week, while a slight decrease in the activity occurred at longer periods (40 days) of treatment. In our studies the pattern of subcellular distribution of the enzyme was unaltered after KI treatment indicating that the decrease observed in the L fraction specific activity reflected a true effect and not a shift of the enzyme to other subcellular site. The fact that S specific activity did not change might be explained on the basis of a parallel effect of KI on both enzyme and on the protein content of this fraction.

KI failed to inhibit the in vitro protease activity. Thus a direct effect on the enzyme or its binding to the substrate is unlikely. These results strongly suggest the assumption that KI interferes either with the enzyme synthesis or turnover. Regarding the first possibility and taking into account the protein nature of the protease, previous reports have demonstrated a KI inhibition of protein biosynthesis (Katakai et al. 1966; Pisarev & Itoiz 1972).

Hence an action of KI on the enzyme synthesis does not appear unlikely. The exact site for KI inhibition is not known and is currently under study.

The action of relatively high concentrations (10⁻² M) of KI on acid protease is striking. These results were observed with enzymes obtained from thyroidal S and LM fractions and also from liver. Thus organ specificity is excluded. It is very difficult to explain the mechanism of this phenomenon. This effect was observed only with KI and not with NaCl or NaF indicating that only the former had the capacity to do so.

Previous results have shown that KI administration caused a decrease of the release of thyroid hormones both in human subjects (Solomon 1956; Greer & DeGroot 1956) and in animals (Ochi & DeGroot 1969; Pisarev et al. 1971). The present results might provide an explanation for this effect, although they do not exclude the existence of other sites of action. It has been observed that there is a delay in the action of KI on hormone release. KI must be administered 24 h before the McKenzie mouse test in order to obtain an inhibition of TSH or cyclic AMP stimulation (Pisarev & DeGroot, unpublished). Similar results were observed in these studies when KI was administered to rats 20 and 4 h before sacrifice and before protease activity was assayed, in agreement with the results of Takeuchi et al. (1970).
ACKNOWLEDGMENTS

The authors would like to thank Prof. J. E. Dumont for helpful discussion and Miss D. Legrand for the preparation of the manuscript.

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Received on March 21st, 1973.