CHOLERA TOXIN: RADIO-IODINATION AND UPTAKE BY THE INTESTINE OF SUCKLING RATS

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Abstract—1. The chloramine-T procedure was employed to radio-iodinate cholera toxin using Na125I. The procedure was found to be efficient and reproducible.

2. Intragastric injections of both the labelled and the unlabelled toxin produced (a) significant increases in intestinal fluid accumulation as measured by the fluid accumulation ratio; (b) significant increases in cAMP levels; and (c) significant decreases in cAMP-phosphodiesterase activities when compared with the controls suggesting that radio-iodination did not impair the biological activity of the toxin.

3. In vivo uptake studies of the labelled toxin by different parts of the intestine indicated that the uptake by the duodenum and jejunum was high and rapid when compared with the ileum implying that there are more binding sites (or receptor proteins) for cholera toxin in the duodenum and jejunum than in the ileum.

INTRODUCTION

The mechanism of intestinal secretion in cholera has been shown to be mediated through the elevation of adenosine 3'-5' cyclic monophosphate (cAMP) and adenylate cyclase activity (Kimberg et al., 1971; Chen et al., 1972; Field, 1974). Recently, cAMP-phosphodiesterase (PDE) has also been reported to be involved in the mechanism of secretion (Aye-Kyaw et al., 1992). In fact, PDE levels were reduced. On the other hand, cholera toxin (CT) induces the intestinal brush border enzyme, alkaline phosphatase, suggesting the possibility of glycoprotein involvement as receptors other than Gm1 gangliosides in the uptake of CT by intestinal cells (Yi-Yi-Myint et al., 1991). It is known that alkaline phosphatase shows varied activity in intestinal epithelial cells all along the intestine (Euknauer and Raffer, 1978). Thus, the present study aimed to label the CT with radio-iodine and investigate whether radio-iodination impaired the biological activity of the toxin and then study the uptake of the iodinated toxin by different segments of the intestine.

MATERIALS AND METHODS

Radio-iodination

The radio-iodination of cholera toxin (purchased from Sigma) was carried out by the chloramine-T procedure as described by Bolton (1977) using Na125I (obtained from The Radiochemical Centre, Amersham).

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Experimental secretory diarrhoea

The procedure has been described previously (Aye-Kyaw et al., 1988, 1992). In brief, seven suckling Wistar rats as controls were administered 0.1 ml of 0.8% (w/v) Evans blue dye in normal saline by intragastric injection. Seven Wistar rats, which constituted the "cold CT treated group" were given 0.1 ml unlabelled CT (50 pg/ml) prepared in the same dye solution. Another five Wistar rats which constituted the "hot CT treated group" were given 0.1 ml radio-labelled CT (50 pg/ml) solution. The milk-filled stomach of the rats was identified below the left lung and between the liver and the spleen as a white patch. After 3 hr, the animals were sacrificed by neck/head dislocation and the stomachs were examined to confirm the existence of dye in the gut lumen. The intestines were removed, and the weights of the gut and the remaining carcass were measured. For each rat, the ratio of gut weight to carcass weight was calculated and a ratio of more than 0.065 was taken as showing a positive effect (i.e. demonstrating a toxic intestinal secretory diarrhoea), according to Dean et al. (1972) and Baselski et al. (1977). All animals in the control group showed ratios of less than 0.065. The intestines were washed with chilled 0.25 M sucrose containing 0.5% of Tris–HCl buffer (pH 7.4), blotted, weighed and then homogenized in 0.25 M sucrose (20 x the weight of tissue) ml with a Potter–Elvehjem homogenizer. Each homogenate was divided into two portions. One portion was frozen and thawed five times to lyse the cells and then used for PDE measurement. The other portion which was used for measuring cAMP levels was heated in a boiling water bath for 2 min to destroy the PDE activity and then immediately frozen and stored at −20°C until the assay.
cAMP, PDE and protein assays

cAMP was assayed using the assay kit supplied by Amersham, U.K. PDE (EC 3.1.4.1) was assayed according to the method described by Lin et al., (1974). The inorganic phosphate released after addition of nucleotidase was measured by the method of Aye-Kyaw et al., (1985). Protein was determined by the method of Miller (1959).

PDE activities were expressed as units (U) per mg protein or per g tissue, where 1 U is equal to 1 μ mole of inorganic phosphate produced per hour. cAMP levels were also expressed as picomoles per mg protein or per g tissue.

Statistical analyses were performed using Student's t-test.

Comparison of in vitro and in vivo uptake

For in vitro study, three suckling Wistar rats were sacrificed by neck head dislocation. For each rat, the abdomen was opened and the whole small intestine was removed. After exposing the mucosa by cutting longitudinally, the intestine was then cut into approximately 1 mm pieces. The pieces obtained from the three rats were first equilibrated at 37°C for 5 min in three separate tubes (one tube for each rat) containing oxygenated tyrode solution and then transferred into similarly pre-equilibrated tubes that contained 0.1 ml of radio-labelled CT (50 μg/ml) in 5.0 ml of oxygenated tyrode solution. Duplicate samples from each tube were taken out at 0, 0.5, 1, 2, 3 and 4 hr, washed in chilled tyrode solution, blotted, weighed and counted on a Gamma Counter, Model 201, Albott. The uptake was expressed as counts per min per g tissue.

For in vivo study, 18 suckling Wistar rats were divided into 6 groups, each group consisting of 3 rats. Each rat received intragastrically 0.1 ml of labelled CT (50 μg/ml) prepared in normal saline. Each group of six was sacrificed at intervals of 0, 0.5, 1, 2, 3 and 4 hr post-injection, respectively. After opening the abdomen, the whole small intestine was removed, washed in cold saline, blotted, weighed and counted on the Counter.

Differential in vivo uptake study

Differential uptake by different segments of the intestine was studied as above except that each intestine was divided into duodenum, jejunum and ileum and counted separately.

RESULTS

Three radioiodination experiments were carried out in the present study and they produced the mean percentage iodination yield of 90.1 ± 3.5 (SEM) with a mean specific radioactivity of 2.25 μCi/μg ± 0.18 (SEM). Therefore, the chloramine-T radio-iodination procedure seemed to be efficient and reproducible for iodinating CT.

Table 1. Fluid accumulation ratios of control and test groups

<table>
<thead>
<tr>
<th>Group</th>
<th>FA ratio</th>
</tr>
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<tbody>
<tr>
<td>Control (C)</td>
<td>0.055 ± 0.001</td>
</tr>
<tr>
<td>(n = 7)</td>
<td></td>
</tr>
<tr>
<td>Cold CT (T)</td>
<td>0.079 ± 0.002</td>
</tr>
<tr>
<td>(n = 7)</td>
<td></td>
</tr>
<tr>
<td>Hot CT (IT)</td>
<td>0.082 ± 0.005</td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
</tr>
<tr>
<td>Significance</td>
<td></td>
</tr>
<tr>
<td>C-T</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>C-IT</td>
<td>P &lt; 0.001</td>
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</tbody>
</table>

Table 2 shows the fluid accumulation ratios of control and CT treated groups. All the animals in both the cold CT and hot CT groups showed fluid accumulation ratios greater than 0.065 and the extent of increase in both the groups is almost the same suggesting that the iodination did not impair the biological activity of CT.

Table 2 shows the cAMP and PDE levels in the control and CT treated groups. PDE activities were significantly decreased in both the cold CT and hot CT groups when compared with the control expressing the enzyme activity per protein or wet tissue weights. Similarly, the levels of cAMP were significantly increased in both the cold CT and hot CT groups on comparison with the control. The results suggest that the mechanism of secretion triggered by CT was not altered by radio-iodination.

Table 1 shows the comparison of in vivo and in vitro uptakes of labelled CT by the intestinal cells. The in vivo uptake and metabolism appeared to be more rapid than in vitro, suggesting that the in vitro model cannot be used for the study of CT uptake by intestinal cells.

The in vivo uptake of labelled CT by different segments of the intestine, namely duodenum, jejunum and ileum (Fig. 2) showed that the uptake by the duodenum and jejunum is higher and quicker than that by the ileum. However, comparing duodenum and jejunum, the uptake appeared to be greater and

Table 2. Intestinal PDE and cAMP levels in control and test groups

<table>
<thead>
<tr>
<th>Group</th>
<th>PDE Units per mg protein</th>
<th>Units per g tissue</th>
<th>pmoles per mg protein</th>
<th>pmoles per g tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>1.04 ± 0.19</td>
<td>31.0 ± 1.40</td>
<td>0.44 ± 0.10</td>
<td>7.19 ± 1.00</td>
</tr>
<tr>
<td>(n = 7)</td>
<td></td>
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<tr>
<td>Cold CT (T)</td>
<td>1.09 ± 0.09</td>
<td>18.2 ± 0.75</td>
<td>1.42 ± 0.30</td>
<td>22.92 ± 4.02</td>
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<tr>
<td>(n = 7)</td>
<td></td>
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<tr>
<td>Hot CT (IT)</td>
<td>1.00 ± 0.09</td>
<td>15.8 ± 0.76</td>
<td>1.16 ± 0.29</td>
<td>25.28 ± 4.35</td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significance</td>
<td></td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.02</td>
</tr>
<tr>
<td>C-T</td>
<td></td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM. Units for PDE are expressed as described under Materials and Methods. 'n' denotes the number of animals.
Intestinal uptake of CT

Fig. 1. Time course of in vitro and in vivo uptakes of labelled CT by the intestine of suckling rats. Each value represents the mean ± SE for 3 rats.

more sustainable in the jejunum rather than in the duodenum.

DISCUSSION

Radio-iodination by the chloramine-T procedure has been generally used for tracer studies in biological systems. The yield and the specific radioactivity obtained in the present study are comparable with those obtained by Hyun and Kimich (1984). Moreover, the iodination procedure seemed not to impair the biological activity and secretory mechanisms of CT since the unlabelled and the labelled CT produced apparently the same responses in fluid accumulation ratios, PDE and cAMP levels.

With regard to deriving an in vitro model for the uptake study of CT by the intestine, the marked difference between in vitro and in vivo results (Fig. 1) indicated that the in vitro model cannot be extrapolated to in vivo conditions.

The higher uptake of CT and sustainability in the jejunum rather than ileum is consistent with the findings of Banwell et al. (1970), who found that in human cholera the fluid loss into the intestinal lumen from the jejunum was greater than that from the ileum. The higher uptake and sustainability may suggest the presence of a large number of toxin binding sites and a relatively slow turnover of the receptor molecules, which are hypothetically GM1 gangliosides (Holmgren et al., 1975; Hyun and Kimich, 1984). Conversely, it is clear that physiologically the brush border must be a target for toxin binding and penetration and indeed work with isolated rat intestinal brush borders confirms this prediction (Crichtley et al., 1981). Furthermore, Yi-Yi-Myiint et al. (1991) reported that CT could interact with the brush border membrane enzyme, alkaline phosphatase. With this view, it may be possible that the preferential uptake of CT by the duodenum and jejunum rather than by the ileum found in the present study is consistent with the fact that in suckling rats, the majority of alkaline phosphatase is membrane bound in the proximal half of the intestine whereas in the ileum the alkaline phosphatase is mostly associated with the supernatant fraction of the homogenate (Yedin et al., 1981). Moreover, it should be noted that alkaline phosphatase is a glycoprotein enzyme and glycoproteins have been suggested as possible receptors to CT other than GM1 gangliosides (Morita et al., 1980).

Apart from above, it may be of interest to note that in the case of E. coli heat stable enterotoxin, which triggers a different secretory mechanism, namely cGMP, an opposite finding was observed, that is, the rat jejunal secretory response to the enterotoxin was brief while the ileal response was sustained (Cohen et al., 1989).

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REFERENCES


