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Intestinal absorption enhancement by sodium taurodihydrofusidate of a peptide hormone analogue (dDAVP) and a macromolecule (BSA) in vitro and in vivo

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Summary

The intestinal absorption enhancing effect of sodium taurodihydrofusidate (STDHF), a bile-salt like compound, was investigated using a nonapeptide hormone analogue, dDAVP, and the protein marker bovine serum albumin (BSA). In vitro, absorption was studied using everted small intestinal sacs. The presence of 15 mmol/l STDHF facilitated mucosal to serosal transport of both dDAVP and BSA (molar ratio STDHF:BSA/dDAVP, 1 : 10). The uptake of dDAVP was highest in the distal (ileal) part of the small intestine. In vivo, dDAVP was administered intragastrically together with BSA and bovine immunoglobulin (BIgG) to pre- and post-closure (14- and 30-day-old) rats. Addition of STDHF to the marker solution increased absorption of dDAVP into circulation in both age groups, while BSA absorption enhancement could only be clearly demonstrated in pre-closure rats. For BIgG, which is absorbed from the intestinal lumen by receptor-mediated processes a decreased absorption was observed when STDHF was present in the pre-closure rats. It is concluded that an agent like STDHF might provide an efficient way to increase transepithelial absorption of larger peptides. At the same time one should be aware of the potential risk of a non-specific mucosal co-transport of intestinal contents when using STDHF.

Introduction

The prospect of delivering peptide hormones and analogues by routes other than the intravenous and subcutaneous pathways is currently attracting much interest (Longenecker and Eppstein, 1988). One such peptide administered both nasally and orally is the vasopressin agonist dDAVP. The effectiveness of orally administered dDAVP in reducing urine output and increasing urine osmolality has been established in both hydrated human volunteers (Vilhardt and Bie, 1984) and diabetes insipidus patients (Hammer and Vilhardt, 1985). Due to the high antidiuretic potency of this drug a bioavailability of less than 1% was considered to be acceptable by the oral route (Vilhardt and Lundin, 1986a).

Another approach to facilitating epithelial absorption would be to use an absorption-promoting agent. Examples of such enhancers include bile

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salts (Duchateau et al., 1986), chelating agents (Yamashita et al., 1985), surfactants (Hirai et al., 1981), and fatty acids (Muranishi, 1985). An ideal absorption enhancer should transiently increase permeability of the mucosal epithelium without causing toxic effects or irreversible damage to the mucosa. One of the most promising agents found to date is sodium taurodihydrofusidate (STDHF) a derivative of fusidic acid which was originally developed as an antibiotic. Recently, STDHF was used to assess the intranasal absorption enhancement of insulin in sheep (Longenecker et al., 1987).

The purpose of the present investigation was to determine whether STDHF could facilitate the transepithelial intestinal transport of dDAVP and the macromolecular markers bovine serum albumin (BSA) and bovine immunoglobulin (BIgG). This was performed both in vitro using everted intestinal sacs and in vivo in young rats by intragastric administration before and after macromolecular 'closure'.

Materials and Methods

dDAVP with a chromatographic purity of greater than 99% was obtained from Ferring Pharmaceuticals (Malmoe). Sodium tauro-24,25-dihydrofusidate (STDHF) was obtained from KARO BIO AB (Huddinge).

Animals

Sprague-Dawley rats (Alab, Stockholm), were kept in our laboratory under a 12 h day-night rhythm at 20 °C with a relative humidity of 50% and had free access to rat chow (R3, Alab) and tap water. Rat pups were kept with their dams until weaning at the age of 21 days.

Viability of intestinal preparation

Although validation experiments have been carried out previously (Vilhardt and Lundin, 1986b), we determined active transport of methyl-D-glucose, 3-*O*-[*methyl*-^3H]methyl-D-glucose across the everted sacs (method described below). The isotope was purchased from New England Nuclear (DuPont de Nemours, F.R.G.) with a specific activity of 2023 GBq/mmol; 15.3 nmol/l (1.18 mCi/l) was added to the mucosal side. Samples were removed from the serosal side at various time points during a 2 h period and transferred to 10 ml scintillation cocktail (Opti Phase 'Hi Safe', LKB, Bromma) for determination of radioactivity on a liquid scintillation counter (LKB). In a separate series, labelled methyl-D-glucose was added to the mucosal side after 10 min preincubation with 2 mM ouabain (Serva, Heidelberg).

In vitro experiment

Male and female rats weighing 220–400 g were fasted overnight. The animals were anaesthetized and subsequently killed with diethyl ether, the abdominal cavity was opened and the small intestine, starting at the end of the duodenum, was removed. Three 5-cm segments were cut from the proximal and distal parts, respectively. The segments were everted, using a stainless-steel rod, and washed in incubation buffer at room temperature. The distal end of each segment was ligated to the cone-shaped tip of the lid and the proximal end was closed by ligation using a modified version of the organ bath described previously (Vilhardt and Lundin, 1986b) (Fig. 1). The organ bath was filled with 8.5 ml incubation medium containing (in mmol/l): NaCl, 110; KCl, 5.5; $CaCl_2$, 3.0; KH_2PO_4, 1.4; $NaHCO_3$, 29; sodium pyruvate, 5.7; sodium fumarate, 7.0; sodium glutamate, 5.7; glucose, 13.4. The medium was gassed with 5% CO_2 in O_2 and kept at 37 °C. The

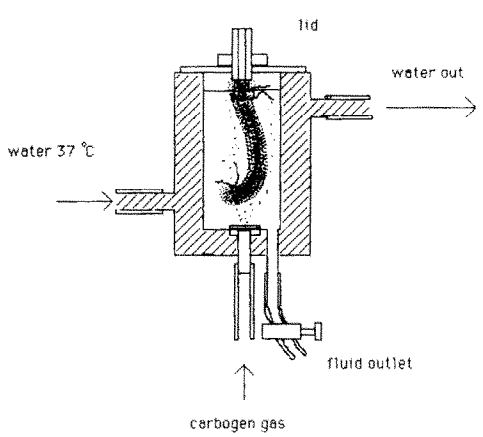

Fig. 1. Diagram of organ bath used for in vitro intestinal transport experiments.

intestinal sacs were filled with 500 μl incubation medium at the serosal side.

The marker, alone or in combination with STDHF, was added to the medium on the mucosal side at the following concentrations: dDAVP (10 nmol/l) + BSA (0.15 mmol/l), with or without STDHF (1.5 or 15 mmol/l) (molar ratios STDHF/dDAVP + BSA, 1:10 and 1:100). At 15, 30, 45 and 60 min, 30-μl samples were removed from the serosal medium and 5 μl placed in 100 μl of 0.1 M HCl for dDAVP analysis. The samples were stored frozen at −20 °C until analyses.

In vivo experiment

Four 30-day-old litters were fasted for 3 h and four 14-day-old litters were separated from their dams 2 h before experiments. The pups were gavage fed by stomach tube 0.05 ml/g body weight of a marker solution containing: dDAVP, 2 μmol/l (100 nmol/kg); BSA, 0.36 mmol/l (1.8×10^{-5} mol/kg) and BIgG 6.7×10^{-5} mol/l (3.3×10^{-6} mol/kg). Half of the rats in a litter received markers only while the other half received the markers together with STDHF, 4.2 mmol/l (2.1 mmol/kg). At 1 or 4 h after feeding, the animals were anaesthetized with mebumal (Nord Vacc, Stockholm; 60 mg/kg body weight i.p.). Blood obtained by heart puncture was allowed to clot at 20 °C and, after centrifugation at 3000 × *g* for 10 min at 4 °C, the serum was aspirated and kept frozen at −20 °C until analysis.

Analyses of markers

A specific radioimmunoassay (RIA) was used for the measurement of dDAVP (Lundin et al., 1985). For in vitro samples direct measurements of 2.5-μl aliquots were performed, while serum samples were extracted prior to analysis. Inter- and intraassay coefficients of variation were 18 and 8.1%, respectively. STDHF assayed alone did not affect binding characteristics of the antiserum.

Quantitation of BSA was performed by electroimmunoassay (Laurell, 1972) with BSA (A-7638, Sigma) as standard and using a specific antiserum to BSA (Dakopatts AB, Hägersten, Sweden). The BIgG concentration was estimated using single radial immunodiffusion (Ingild, 1983) with BIgG (63500, Sigma) as standard and a specific antiserum to BIgG (Miles Laboratories, Naperville, U.S.A.). Statistical calculations were performed using Student's *t*-test and analysis of variance (ANOVA).

Results

In order to assess the viability of the in vitro intestinal preparation, the actively transported sugar [^{3}H]methyl-D-glucose was used. The mucosal-to-serosal transport was nearly linear over the 2 h incubation period (Fig. 2). Addition to the medium of 2 mM ouabain significantly reduced transport after 60 min ($p < 0.001$).

The in vitro experiments revealed that the transmucosal absorption of dDAVP was generally lower in the proximal than in the distal intestine ($p < 0.001$) (Fig. 3). Addition to the medium of 1.5 and 15 mM STDHF significantly increased intestinal absorption of dDAVP ($p < 0.01$ and < 0.001, respectively by two-way ANOVA) in the distal segment. In the proximal segments a significantly increased absorption could be demonstrated using 15 mM STDHF ($p < 0.001$). After 60 min incubation the serosal dDAVP concentration was 3–7% of that on the mucosal side. The

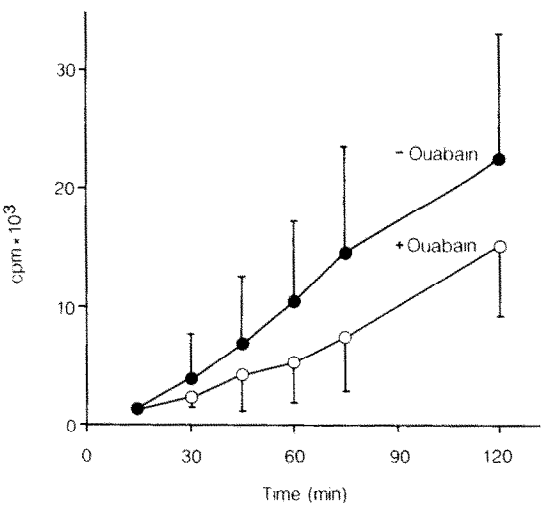

Fig. 2. Mucosal-to-serosal transport of [^{3}H]methyl-*O*-glucose measured as cpm across everted rat intestinal sacs in the presence and absence of 2 mM ouabain (means ± S.D., $n = 6$).

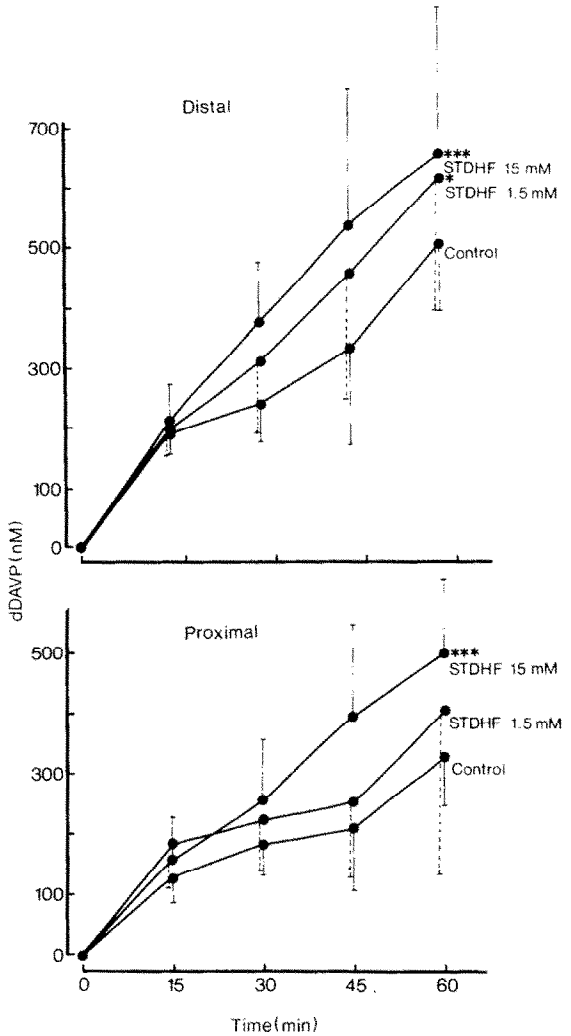

Fig. 3. Mucosal-to-serosal passage of dDAVP with time after incubation of everted proximal and distal segments taken from the small intestine in a medium containing 10 mM dDAVP without (control) and with STDHF present (1.5 and 15 mM). Values are means ± S.D., $n = 7$. Significant differences from control were obtained by two-way ANOVA as indicated by * $p < 0.01$ and *** $p < 0.001$.

absorption of BSA added to the mucosal medium in the presence of 15 mmol/l STDHF was enhanced ($p < 0.001$) while the lower concentration of STDHF was ineffective (Fig. 4). In contrast to dDAVP, BSA absorption deviated from linearity at 60 min. The concentration of BSA in the serosal medium at 60 min was 0.1% of that in the mucosal medium.

In vivo the serum levels of dDAVP were much higher in pre-closure (14 days) than in post-closure (30 days) rats (Fig. 5a–b). When 4.2 mmol/l STDHF was administered together with the markers the serum levels of dDAVP were significantly elevated in both age groups. BSA serum levels were large only in 14-day-old rats, where STDHF induced several-fold enhancement of the BSA levels (Fig. 6). In post-closure rats BSA could be measured only at low levels in a few samples. The in vivo intestinal absorption of BIgG in pre-closure rats was hampered by the presence of STDHF which caused a 2-fold reduction in serum

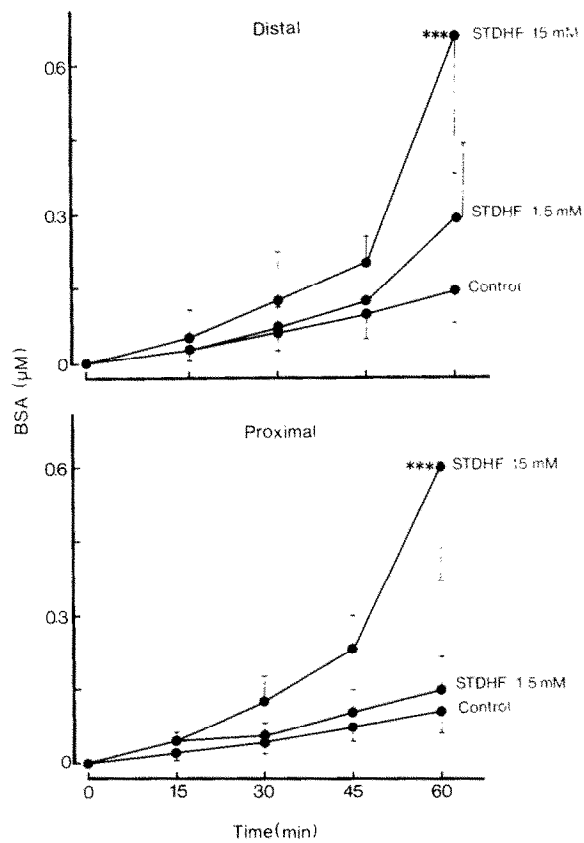

Fig. 4. Mucosal-to-serosal passage of BSA with time after incubation of everted proximal and distal segments taken from the small intestine in a medium containing 10 mM dDAVP without (control) and with STDHF present (1.5 and 15 mM). Values are means ± S.D., $n = 7$. Significant differences from the control were obtained by two-way ANOVA as indicated by * $p < 0.05$ and *** $p < 0.001$.

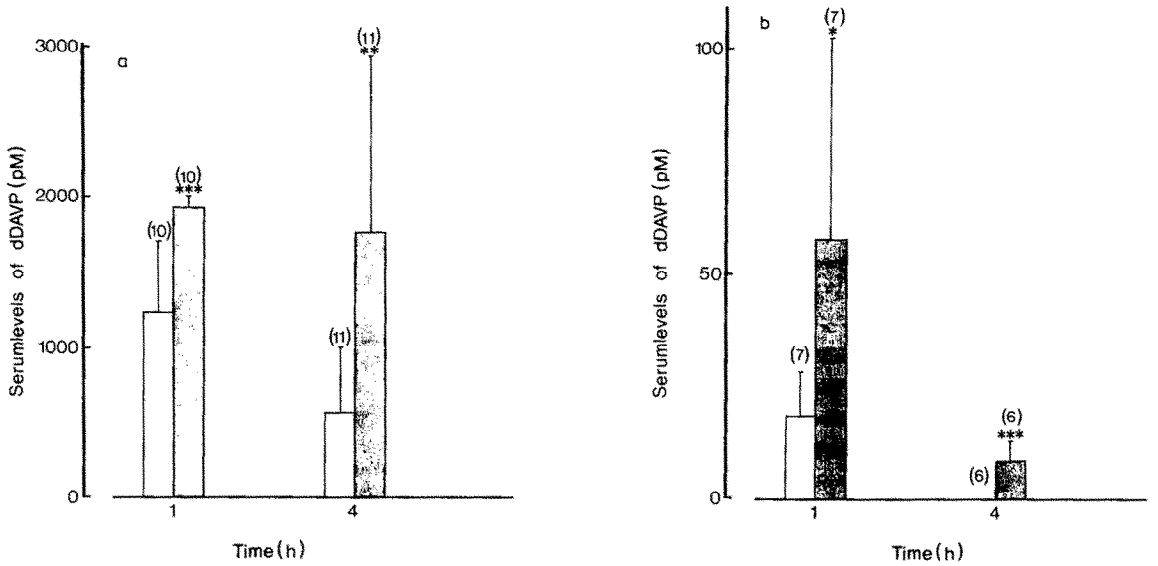

Fig. 5. (a) Serum levels of dDAVP in 14-day-old rats 1 and 4 h after gavage feeding of dDAVP (100 nmol/kg) without (open bars) and with (shaded bars) STDHF (2.1 mmol/kg). Statistically significant difference from control is given by ** $p < 0.05$, *** $p < 0.001$. Values are given as means ± S.D. Numbers of animals are shown in parentheses above the bars. (b) Serum levels of dDAVP in 30-day-old rats 1 and 4 h after gavaged feeding with dDAVP as in panel a.

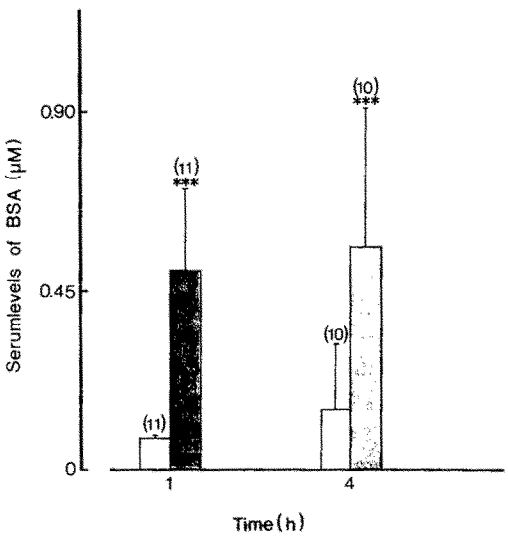

Fig. 6. Serum levels of BSA in 14-day-old rats 1 and 4 h after gavage feeding BSA (1.8×10^{-5} mol/kg) with (shaded bars) and without (open bars) STDHF (2.1 mmol/kg). Statistically significant difference from control is given by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Values are given as means ± S.D. Number of animals are shown in parentheses above the bars.

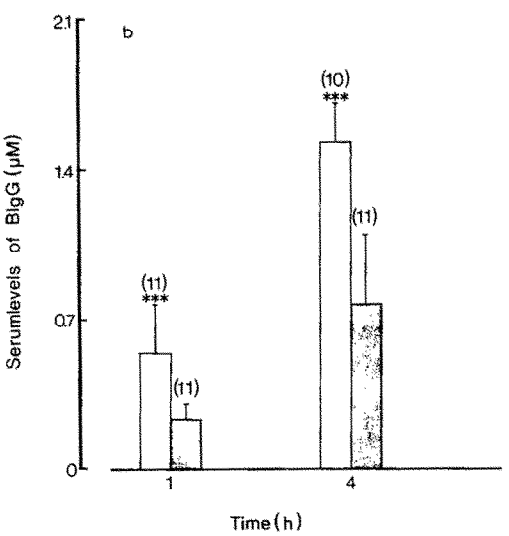

Fig. 7. Serum levels of BIgG in 14-day-old rats 1 and 4 h after gavage feeding BIgG with (shaded bars) and without (open bars) STDHF (2.1 mmol/kg). Statistically significant difference from control is given by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Values are given as means ± S.D. Number of animals are shown in parentheses above the bars.

levels at 1 and 4 h after administration of markers (Fig. 7).

Discussion

The use and validity of the everted sac model to study intestinal transport of large peptides has previously been described (Vilhardt and Lundin, 1986b). Apart from being simple it should also be reliable. Further validation of this model was achieved by studying the transport of ^{3}H-labelled methyl-D-glucose which is absorbed by active processes (Takaori et al., 1986; Grass and Sweetana, 1988). The linear transport rate of [^{3}H]methyl-D-glucose and the decrease in transport in the presence of the metabolic inhibitor ouabain suggest that the intestinal preparation remains viable for a period of at least 2 h.

The addition of STDHF increased the transepithelial passage of the peptide dDAVP across small intestine in vitro. Absorption enhancement was also evident using a macromolecule like BSA (69 kDa). The critical micellar concentration of STDHF is 2.5 mM (Longenecker et al., 1987). This may explain why transport enhancement was relatively poor using the lower concentration (1.5 mM) of STDHF in vitro.

The data obtained showed that dDAVP was transported at a higher rate in segments taken from the distal than from the proximal part of the small intestine. This is in agreement with earlier observations in vivo where dDAVP was absorbed to a higher extent from this portion of the intestine in rabbits (Lundin and Vilhardt, 1986). This finding could be attributed to differences in proteolytic activity in various parts of the small intestine. dDAVP has not been shown to be degraded when incubated with intestinal segments (Vilhardt and Lundin, 1986b). However, degradation of dDAVP as assessed by HPLC has been demonstrated using intestinal mucosa homogenates when the peptide is exposed to intracellular enzymes (Lundin et al., 1989). Moreover, intestinal permeability generally was shown to be lower in the distal part of the small intestine for molecules other than peptides (Loehry et al., 1973). Further studies are therefore required in order to elucidate the nature of this increased dDAVP absorption in the distal small intestine. No such difference in regional transport could be found using BSA. One reason for the non-linear appearance of the mucosal-to-serosal transport of BSA may be the slow solubilization of this protein in the presence of STDHF. STDHF seemed to prevent aggregation of insulin monomers (Longenecker et al., 1987).

For all markers the intestinal closure phenomenon was evident at 30 days of age (Walker et al., 1972). Only dDAVP could be detected with certainty in post-closure rats. However, the mechanisms of absorption differ between these molecules. BSA is absorbed by fluid-phase endocytosis (Baintner, 1986) whereas BIgG (150 kDa) is absorbed by Fc receptor-mediated transport (Mackenzie, 1984). The uptake of dDAVP in preclosure rats seems to follow the BSA uptake, indicating similar transport routes (Folkesson et al., 1988). The addition of STDHF to the marker solution also affected the transport of the markers differently. Both dDAVP and BSA were absorbed to a greater extent while BIgG transport was significantly reduced. The reducing effect of STDHF on the receptor-mediated BIgG transport may be due to interference with IgG binding to its Fc receptor on the enterocyte.

The mechanism by which STDHF acts to increase mucosal permeability is not understood. Intranasal absorption enhancement of insulin by STDHF was proposed to occur by the formation of mixed micelles of STDHF and insulin (Longenecker et al., 1987). Another important mechanism of this compound could be inhibition of mucosal proteases by denaturing these enzymes (Gallardo et al., 1987). Entrapment of peptide or protein in micelles of STDHF and other enhancers may also lead to protection against proteolysis, a possibility that needs to be further explored.

In conclusion, STDHF administered together with the peptide hormone analogue dDAVP and the macromolecule BSA leads to an improvement in intestinal permeability. After macromolecular closure, permeability enhancement could be demonstrated for dDAVP alone. The addition of an agent like STDHF claimed to be of low toxicity (Longenecker et al., 1987) to formulations of

peptide drugs could make oral administration a feasible route, although one has to consider the possibility of an increased non-specific uptake of cotransported intestinal contents.

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