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Ethanol-induced modification of somatostatin-responsive adenylyl cyclase in rat exocrine pancreas

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Abstract

Male rats were given 10% (w/v) ethanol in drinking fluid during the first week, 15% (w/v) during the second week, 20% (w/v) during the third, and 25% (w/v) during the fourth week, at the end of which they were kept on 25% (w/v) ethanol drinking water for 3 weeks. Some animals were then allowed the withdrawal of ethanol for a period of 2 weeks or 7 weeks. No significant differences were seen for the basal and forskolin (FK)-stimulated adenylate cyclase (AC) enzyme activities in the pancreatic acinar membranes of ethanol-treated and ethanol withdrawal rats as compared to the control group. Chronic ethanol ingestion resulted in an attenuation of somatostatin(SS)-inhibited FK-stimulated AC in rat pancreatic acinar membranes. The ability of the stable GTP analogue 5′-guanylylimidodiphosphate (Gpp[NH]p) to inhibit FK-stimulated AC activity was also decreased in pancreatic acinar membranes from ethanol-treated rats. Gpp[NH]p was a much less potent inhibitor of SS binding in the pancreatic acinar membranes from chronic ethanol-treated animals than in those from controls, suggesting a change of G_i. A significant reduction in the number of ^{125}I-Tyr^{11}-SS receptors was observed after ethanol ingestion, when compared with control values. Two weeks after the replacement of the ethanol solution by water, the ethanol effect on the parameters cited above persisted. At week 7 of withdrawal, these parameters reached the level of water controls. Ethanol administration did not affect either the number or the affinity of secretin receptors as compared to control values which suggests that the change in SS binding is not a non-specific effect. Neither chronic ethanol consumption nor withdrawal affected somatostatin-like immunoreactivity (SSLI). These results suggest that the attenuated inhibition of AC by SS in pancreatic acinar membranes from ethanol-treated rats and ethanol withdrawal (2 weeks) rats may be caused by decreases in both G_i activity and in the number of SS receptors. Alternatively, an uncoupling of SS receptors from G_i and/or a decrease in the level of functional G_i may result in both a decrease in apparent B_{max} for SS binding and in SS-mediated inhibition of AC. Since SS has been suggested to be an inhibitor of basal and cholecystokinin (CCK)- and/or secretin-stimulated exocrine pancreatic secretion, it is tempting to speculate that the impairment of the SS receptor/effector system seen in the present study can participate in the increase of basal pancreatic exocrine secretion described after chronic ethanol consumption.

Keywords: Ethanol; Somatostatin receptor; Adenylyl cyclase; G protein

1. Introduction

Somatostatin (SS), a tetradecapeptide initially isolated from the hypothalamus as an inhibitor of pituitary growth hormone secretion [1], functions as a local regulator in the pancreas and gut [2]. It has been previously reported that pancreatic acini possess specific binding sites for SS [3–5], the binding of which results in the triggering of some biological actions in the pancreas [6]. In pancreatic acinar cells, SS receptors are coupled to the adenylate cyclase (AC) enzyme system via the guanine nucleotide inhibitory protein G_i [3,4]. The extent of G_i modifications is correlated with the ability of SS to inhibit adenosine 3′-5′-cyclic monophosphate (cAMP) formation [5]. Guanine nucleotides also inhibit SS binding to its receptor [4,5,7]. Among its various inhibitory functions [6], SS decreases pancreatic exocrine secretion [8,9].

Previous studies have shown that ethanol can modify the action of various peptide hormones on AC in exocrine pancreas [10] and in other tissues [11,12]. Ethanol has also recently been suggested to affect G_i [13]. Since SS inhibits FK-stimulated AC via G_i [5], ethanol consumption may modify the SS mechanism of action in the exocrine pan-

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creas. The goals of this study were to determine if chronic ethanol ingestion could alter the ability of SS to inhibit FK-stimulated AC in rat pancreatic acinar membranes and to explore the possible steps at which these alterations might occur. In addition, the effect of withdrawal, at 2 and 7 weeks after the cessation of ethanol ingestion was also studied. We have measured the overall catalytic activity of the AC activity using FK, an agent which potently stimulates the catalytic subunit [14]. Experiments were also performed to measure the inhibition of specific ^{125}I-Tyr^{11}-SS binding by the stable GTP analogue, Gpp[NH]p in order to determine the integrity of SS receptor binding site-G protein interactions. In addition, the density of ^{125}I-Tyr^{11}-SS binding sites and SS-mediated AC activity in pancreatic acinar membranes were compared and the somatostatin-like immunoreactivity (SSLI) content in pancreas from control and ethanol-treated rats was determined.

2. Materials and methods

2.1. Materials

Synthetic ^{11}Tyr-SS was purchased from Universal Biologicals (Cambridge, UK); secretin porcine was from Neosystem Laboratoire (Strasbourg, France); carrier-free Na^{125}I (IMS 30, 100 mCi/ml) was purchased from the Radiochemical Center (Amersham, UK); forskolin, bacitracin, phenylmethylsulphonylfluoride (PMSF), guanosine triphosphate (GTP), Gpp[NH]p, 3-isobutyl-1-methylxantine (IBMX) and bovine serum albumin (BSA) were purchased from Sigma (St Louis, MO, USA). The rabbit antibody used in the radioimmunoassay technique was purchased from the Radiochemical Centre (Amersham, UK). This antiserum was raised in rabbits against SS-14 conjugated to BSA and is specific for SS. Since SS-14 constitutes the C-terminal portions of both SS-25 and SS-28, the antiserum does not distinguish between these three forms. The binding of SS-14 to this antibody does not depend on an intact disulfide bond in the molecule, since breaking of the disulfide bond by reaction with 0.1% mercaptoethanol (boiling water bath, 5 min) did not change peptide immunoreactivity. Cross-reactivity with other peptides was less than 0.5%. Cross-reaction with several SS analogues demonstrated that neither the N-terminal glycine nor the C-terminal cysteine residues are required for antibody binding, suggesting that the antigen site is directed towards the central part of the molecule containing the tryptophan residue. All other reagents were of the highest purity commercially available.

2.2. Experimental animals

Male Sprague–Dawley rats ($n = 25$) from our own colony, weighing 200 ± 10 g at the beginning of the experiment, were maintained under conditions of automatically controlled temperature (25 ± 1° C) and 12 h light/12 dark cycles (09.00–21.00 h). Animals were fed Purina chow (UAR-Panlab, Barcelona, Spain) ad libitum. Coprophagia was avoided by placing wire nets over the cage floor. Daily caloric intake was estimated from the specific calorific value of the commercial Purina chow used (3200 cal/kg) and the amount of food consumed by the animals, which was determined by daily weighing of offered and remaining food. The amount of daily fluid intake was also determined by volume difference between the offered and remaining liquid, and ethanol calories were estimated as 7.1 cal/g. Animals were subjected to chronic ethanol administration in their drinking fluid as described by Testar et al. [15]. Alcohol-treated rats were given 10% (w/v) ethanol in drinking fluid for 1 week, 15% (w/v) ethanol during the second week, 20% (w/v) ethanol during the third week and 25% (w/v) ethanol during the fourth week. At the end of week 4 the animals were kept on 25% (w/v) ethanol in drinking water for 3 weeks. Control rats received no treatment but were handled in the same way as the ethanol-treated animals. The effects of withdrawing ethanol from chronically treated animals were studied by withholding ethanol for 2 and 7 weeks. The pancreas was removed and trimmed free of fat, connective tissues, and lymph nodes.

2.3. Blood ethanol levels

Rats were decapited and blood samples were collected from the neck wound into heparinized receptacles for immediate plasma separation. Plasma aliquots of 0.5 ml were used for determination of ethanol by head space gas chromatography as previously described [16], using a Perkin-Elmer gas chromatograph (model Sigma 3B) equipped with a flame ionization detector, a head-space injection device, and a Sigma 15 integrator and recorder.

2.4. Preparation of rat pancreatic acinar membranes

Dispersed pancreatic acini were obtained from male Wistar rats after enzymatic degradation of the organ with 0.2 units of collagenase/ml in an oxygenated Krebs-Ringer medium as described by Amsterdam et al. [17]. After thorough washing by sedimentation, acini were transferred to 0.3 M sucrose. In 0.3 M sucrose the acini were homogenized at 4° C by use of a Potter homogenizer following the Meldolesi et al. method [18]. After sedimentation at 1500 $\times g$ for 12 min, the homogenized membranes were resuspended in 1.56 M sucrose. This suspension was overlaid with 0.3 M sucrose and centrifuged at 105 000 $\times g$ for 150 min. The plasma-membrane-enriched fraction collected from the interphase was pelleted and stored at −70° C.

2.5. Binding of ^{125}I-Tyr^{11}-SS

Tyr^{11}-SS was radioiodinated by chloramine-T iodination according to the method of Greenwood [19]. Separa-

tion of iodinated SS from unincorporated iodine was carried out on a Sephadex G-25 (fine) column equilibrated and eluted with 0.1 M acetic acid in BSA (0.1% w/v). The specific activity of the radioligand was 600 Ci/mmol.

Binding of ^{125}I-Tyr^{11}-SS was assayed on pancreatic acinar membranes from rats by a modified method [20]. Binding of ^{125}I-Tyr^{11}-SS to pancreatic acinar membranes was carried out in a total volume of 250 μl in 50 mM Tris-HCl buffer (pH 7.4) containing 0.5 mM $MgCl_2$, 3mM NaCl, 0.2 mM $CaCl_2$, 0.2% (wt/vol) BSA, 0.5 mg/ml bacitracin and 0.3 mg/ml soybean trypsin inhibitor (binding buffer). Plasma membranes, at 75 μg of protein/ml were incubated for 90 min at 20° C with 35 pM ^{125}I-Tyr^{11}-SS in the absence or presence of 0.01–10 nM unlabelled SS. Bound and free ligand was separated by centrifugation at 11 000 × g for 4 min at 4° C in a microcentrifuge. Radioactivity in the pellet was measured with a gamma scintillation counter. Non-specific binding was estimated as membrane-associated radioactivity in the presence of 1 μM SS and specific binding was calculated as the difference between total and non-specific membrane-associated radioactivity. The effects of Gpp[NH]p on ^{125}I-Tyr^{11}-SS binding were determined after addition of a range of Gpp[NH]p concentrations (10^{-11}–10^{-4} M) in the binding assay buffer.

2.6. Evaluation of radiolabelled peptide degradation

To determine the extent of tracer degradation during incubation, we measured the ability of preincubated peptide to bind to fresh pancreatic acinar membranes. Briefly, ^{125}I-Tyr^{11}-SS (35 pM) was incubated with pancreatic acinar membranes (75 μg protein/ml) for 90 min at 20° C. After this preincubation, aliquots of the medium were added to fresh pancreatic acinar membranes and incubated for an additional 90 min at 20° C. The fraction of the added radiolabelled peptide which specifically bound during the second incubation was measured and expressed as a percentage of the binding that had been obtained in control experiments performed in the absence of pancreatic acinar membranes during the preincubation period.

2.7. Binding of ^{125}I-secretin

^{125}I-secretin was prepared by a minor modification of the method of Chang and Chey [21]. ^{125}I-secretin was separated using gel chromatography on Sephadex G-15/G-50, fine (7/3, wt/wt), followed by chromatography on SP-Sephadex C25. The specific activity of ^{125}I-secretin determined with a secretin-specific antiserum was 640–700 Ci/mmol.

Binding of ^{125}I-secretin was assayed on pancreatic acinar membranes from rats by a modified method [22]. The standard binding assay consisted of incubating membranes (150 μg of protein) at 22° C for 30 min in 0.250 ml of 20 mM Tris/HCl/1% albumin buffer (pH 7.4) containing 0.1 mg trypsin inhibitor, 0.05 mg bacitracin, [^{125}]secretin (0.1–0.5.10^{-10} M) and different amounts (10^{-11} M–10^{-6} M) of unlabelled secretin. Bound and free ligand was separated by centrifugation at 11 000 × g for 4 min at 4° C in a microcentrifuge. Radioactivity in the pellet was measured with a gamma scintillation counter.

2.8. Adenylate cyclase assay

AC activity was measured as previously reported [23] with minor modifications. Briefly, rat pancreatic acinar membranes (0.12 mg protein/ml) were incubated with 1.5 mM ATP, 5 mM $MgSO_4$, 1 μM GTP and ATP-regenerating system (7.5 mg/ml creatine phosphate and 1 mg/ml creatine kinase), 1 mM 3-isobutyl-1-methylxantine, 0.1 mM PMSF, 1 mg/ml bacitracin, 1 mM EDTA, and tested substances (10^{-9} M SS or 10^{-5} M FK) in 0.1 ml of 0.025 M triethanolamine/HCl buffer (pH 7.4). After 30 min incubation at 30° C, the reaction was stopped by heating the mixture for 3 min. After refrigeration, 0.2 ml of an alumina slurry (0.75 g/ml in triethanolamine/HCl buffer [pH 7.4]) was added and the suspension centrifuged. The supernatant was taken for assay of cyclic AMP by the method of Gilman [24].

2.9. Tissue extraction and SS radioimmunoassay

For SSLI measurement, the pancreata were rapidly homogenized in 1 ml 2 M acetic acid using a Brinkman polytron (setting 5, 30 s). The extracts were boiled for 5 min in an ice-chilled water-bath, and aliquots (100 μl) were removed for protein determination [25]. The homogenates were subsequently centrifuged at 15 000 × g for 15 min at 4° C and the supernatant was neutralized with 2 M NaOH. The extracts were stored at −70° C until assay. The SS concentration was determined in tissue extracts by a modified radioimmunoassay method [26], with a sensitivity limit of 10 pg/ml. Incubation tubes prepared in duplicate contained 100 μl samples of tissue extracts or standard solutions of 0–500 pg cyclic SS-14 diluted in phosphate buffer (0.05 M [pH 7.2] containing 0.3% BSA, 0.01 M EDTA), 200 μl appropriately diluted anti-SS serum, 100 μl freshly prepared ^{125}I-Tyr^{11}-SS diluted in buffer to give 6000 cpm (equivalent to 5–10 pg), in a final volume of 0.8 ml. All reagents as well as the assay tubes were kept chilled on ice before their incubation for 48 h at 4° C. Bound hormone was separated from free hormone by the addition of 1 ml dextran-coated charcoal (dextran T-70, 0.2% w/v, Pharmacia, Uppsala, Sweden; charcoal: Norit A, 2% w/v Serva, Feinbiochemica, Heidelberg, Germany). Serial dilution curves for the samples were parallel with the standard curve. The intra-assay and inter-assay variation coefficients were 6.0 and 8.8% respectively.

2.10. Data analysis

Binding parameters for SS receptor binding were determined by least squares analysis of Scatchard plots [27]. Statistical comparisons of all the data were carried out with one-way analysis of variance (ANOVA) and the Student/Newman-Keuls test. Means among groups were considered significantly different when the P-value was less than 0.05. Each individual experiment was performed in duplicate.

3. Results

Blood ethanol levels at the time that the rats were killed were 30.1 ± 3.5 mM in chronic ethanol-treated rats and after ethanol withdrawal, the blood ethanol disappeared. With chronic ethanol administration in the drinking fluid, both daily food intake (45.7%) and drinking fluid intake decreased (32.6%) with respect to the start of ethanol administration, and these values were significantly lower than those in control rats. Total energy intake in the ethanol-treated group did not differ from that of the control group because the progressive increment in ethanol-derived calories balanced the correlated decrease in calories ingested by these rats. Animal weights were significantly reduced in the ethanol-treated group (230 ± 4 g) as compared with control rats (314 ± 3 g) from the 14th day of treatment and this difference remained until the end of the chronic study (268 ± 7 g vs. 352 ± 6 g).

No significant differences were seen for either basal or FK-stimulated AC activity between control and ethanol-treated and ethanol withdrawal (2 weeks) rats (Table 1). It should be noted that SS did not modify basal AC activity but inhibited FK-stimulated AC activity in all experimental groups. In the ethanol-treated and ethanol withdrawal (2 weeks) rats, the capacity of SS to inhibit FK-stimulated AC activity was significantly lower than in the control group and returned to control values 7 weeks after ethanol withdrawal (Table 1).

In the presence of FK (10^{-5} M), which amplifies basal AC activity, increasing concentrations of Gpp[NH]p produce an inhibitory effect that has been used by several investigators as a measure of G_i function in membranes prepared from different tissues [28]. Pancreatic acinar membranes from control, ethanol-treated and ethanol withdrawal rats exhibited Gpp[NH]p-elicited inhibitory effects on AC (Fig. 1). However, the inhibition achieved at concentrations of Gpp[NH]p ranging between 10^{-11} and 10^{-6} M was significantly lower in pancreatic acinar membranes from ethanol-treated and ethanol withdrawal (2 weeks) rats than in those from control rats.

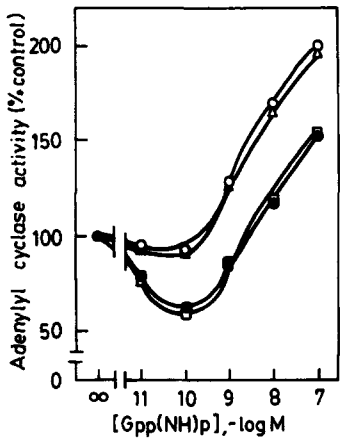

Fig. 1. Dose–effect curves for 5′-guanylylimidodiphosphate (Gpp[NH]p) on forskolin (FK)-stimulated adenylyl cyclase (AC) activity in rat pancreatic acinar membranes from control (●), ethanol-treated (○) and ethanol withdrawal (2 weeks (△) and 7 weeks (□)) rats. Data are expressed as a percentage of FK-stimulated activity (100%) in the absence of Gpp[NH]p. The results are given as the mean ± S.E.M.: of three determinations performed in duplicate. For the sake of clarity, S.E.M. are not represented but were always below 10% of the mean values.

The ability of Gpp[NH]p to inhibit binding of ^{125}I-Tyr^{11}-SS [4,5,7] was used as a functional assay for G_i. Gpp[NH]p was a much less potent inhibitor of binding in the membranes from ethanol-treated and ethanol withdrawal (2 weeks) rats than in those from controls (Fig. 2). The 50% inhibitory concentration for Gpp[NH]p was 0.24 μM in control rats and 0.50, 0.63 and 0.24 μM in rats subjected to chronic ethanol treatment and in rats at 2 and 7 weeks after ethanol withdrawal, respectively. Chronic

Table 1
Effect of somatostatin (SS) (10^{-9} M) and forskolin (10^{-5} M) on adenylyl cyclase (AC) activity (pmol cAMP/min/ mg protein) in pancreatic acinar membranes of control and ethanol-treated rats and ethanol withdrawal (2 and 7 weeks) rats

	Control	Ethanol	Ethanol withdrawal (2 weeks)	Ethanol withdrawal (7 weeks)
Basal activity	13.4 ± 0.6	13.0 ± 0.9	12.8 ± 0.7	13.5 ± 0.5
Basal activity + 10^{-9} M SS	11.9 ± 0.5	12.1 ± 0.7	11.7 ± 0.8	11.8 ± 0.3
+ 10^{-5} M FK	24.3 ± 1.1	25.5 ± 1.3	25.9 ± 1.5	25.1 ± 1.2
Fold FK stimulation over basal	1.8 ± 0.1	1.9 ± 0.2	2.0 ± 0.1	1.8 ± 0.3
10^{-5} M FK + 10^{-9} M SS	17.1 ± 0.4	21.4 ± 0.8	22.0 ± 0.9	17.9 ± 1.1
% SS inhibition of FK stimulation	52.3 ± 3.7	25.6 ± 2,4 *	21.4 ± 2.2 *	47.2 ± 4.7

Experiments were performed as described in Section 2. Values represent the mean ± S.E.M. of five separate experiments. Statistical comparison versus control: * $P < 0.05$.

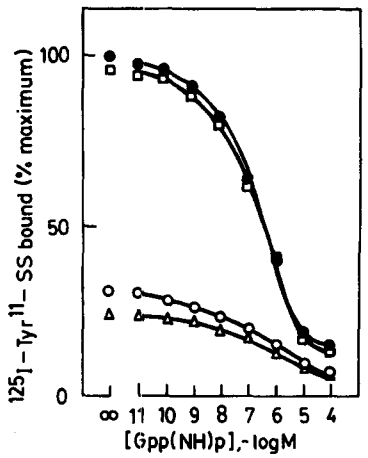

Fig. 2. Dose–effect curves for 5′-guanylylimidodiphosphate (Gpp[NH]p) on the specific binding of [^{125}I]-Tyr^{11}-somatostatin ([^{125}I]-Tyr^{11}-SS 35 pM) to rat pancreatic acinar membranes from control (●), ethanol-treated (○) and ethanol withdrawal (2 weeks (△) and 7 weeks (□)) rats. The results express the values of a pool of the control groups, since differences among them were not found. Each point is the mean of six separate experiments, each performed in duplicate. For the sake of clarity, S.E.M. are not represented but were always below 9% of the mean values. Calculated 50% inhibitory concentration values were 0.24 μM in control rats, 0.50 μM in rats subjected to chronic ethanol administration, and 0.63 μM and 0.24 μM in ethanol withdrawal rats at 2 and 7 weeks.

ethanol treatment increased the potency of Gpp[NH]p 2.08-fold, whereas in ethanol withdrawal rats at 2 weeks the potency was increased 2.62-fold. The potency of Gpp[NH]p in ethanol withdrawal rats at 7 weeks returned to control values.

The specific binding of ^{125}I-Tyr^{11}-SS to pancreatic acinar membranes was time-dependent in all experimental groups. An apparent equilibrium was observed between 60 and 120 min at 20° C (data not shown). All subsequent binding experiments were therefore conducted at 20° C for 90 min. Peptide degradation was determined to rule out the possibility of different SS degrading activities in any of the preparations which might have affected the interpretation of the results. Membranes from each experimental group showed a similar peptide degradation capacity and the values varied by no more than 11% among experimental groups.

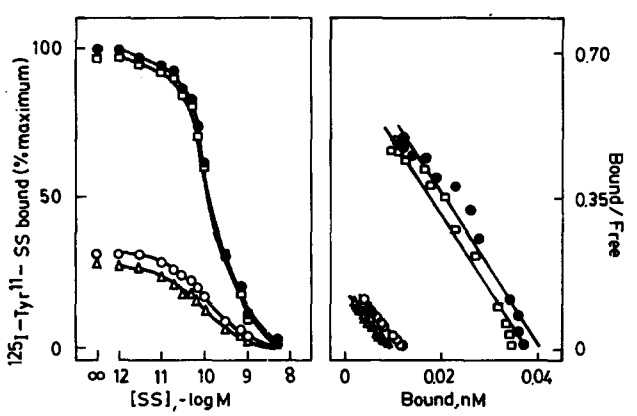

Fig. 3. Left panel: Competitive inhibition of [^{125}I]-Tyr^{11}-somatostatin ([^{125}I]-Tyr^{11}-SS, 35 pM) binding to pancreatic acinar membranes by unlabelled somatostatin (SS). The membranes (75 μg protein/ml) were incubated for 90 min at 20° C in the presence of 35 pM [^{125}I]-Tyr^{11}-SS and increasing concentrations of native peptide. Points correspond to values for control (●), ethanol-treated (○) and ethanol withdrawal (2 weeks (△) and 7 weeks (□)) rats. Each point is the mean of six separate experiments, each performed in duplicate. The results express the value of a pool of the control groups, since the B_{max} and the K_d values of the control groups were not affected. For the sake of clarity, S.E.M.'s are not represented but were always below 10% of the mean values. Right panel: Scatchard analysis of the same data.

Table 2
Effect of chronic ethanol treatment and its withdrawal (2 and 7 weeks) on somatostatin-like immunoreactivity (SSLI) concentration in rat pancreas and on the equilibrium parameters for somatostatin (SS) binding to rat pancreatic acinar membranes

Groups	SS receptors		SSLI
	B_{max}	K_d	
Control	538 ± 18	0.055 ± 0.002	7.69 ± 0.83
Ethanol	171 ± 9 ***	0.061 ± 0.012	6.28 ± 0.92
Ethanol withdrawal (2 weeks)	107 ± 9 ***	0.061 ± 0.006	6.12 ± 0.72
Ethanol withdrawal (7 weeks)	477 ± 20	0.050 ± 0.062	6.66 ± 0.72

Binding parameters were calculated from Scatchard plot by linear regression. Units for SSLI are ng SS per mg protein, units for K_d are nM and units for B_{max} are femtomoles of SS bound per mg of protein. The results are represented as the means ± S.E.M. of five separate experiments. Statistical comparison versus control: *** $P < 0.001$.

Competitive inhibition of ^{125}I-Tyr^{11}-SS binding by increasing concentrations of unlabeled SS was performed in pancreatic acinar membranes from control and ethanol-treated rats (Fig. 3). Chronic ethanol ingestion decreased ^{125}-I-Tyr^{11}-SS binding at all unlabelled SS concentrations. Scatchard analysis of stiochiometric data revealed that ethanol decreased maximal binding capacity and did not modify the affinity of SS for its receptors (Table 2). Two weeks after replacement of the ethanol solution by water, the effect of ethanol on the parameters cited above persisted. At week 7 after withdrawal, these parameters reached the level of water controls.

Neither chronic ethanol consumption nor withdrawal affected SSLI levels (Table 2). Ethanol administration did not affect either the affinity (3.9 ± 0.5 vs 3.7 ± 0.6, nM) or the number (0.8 ± 0.1 vs 0.9 ± 0.2, pmol/mg protein) of secretin receptors as compared to control values.

4. Discussion

In the present study we have reported, for the first time, the effect of chronic ethanol intake on the SSLI content and the SS receptor/effector system in rat pancreatic acinar membranes. In the experimental protocol used in this study, the percentage of ethanol-derived calories and blood ethanol levels were similar to those reported when ethanol was given in a liquid diet [29–32]. Similar concen-

trations of ethanol have been detected in the blood of humans with severe intoxication [33].

The observations of other authors showing that SS does not modify basal AC activity [3,34] and that FK stimulates rat pancreatic AC activity [35] are confirmed in the present study. SS was a partial antagonist of FK-stimulated pancreatic AC activity, in agreement with Heisler [34]. The ability of SS to inhibit FK-stimulated AC is dramatically attenuated in pancreatic acinar membranes from ethanol-treated and ethanol withdrawal (2 weeks) rats compared with their controls. However, there did not appear to be any defect in the catalytic unit of AC itself since in membranes from either control, ethanol-treated and ethanol withdrawal (2 weeks) animals, similar levels of activity were noted both in basal conditions and when this enzyme was directly stimulated by the diterpene FK.

The inhibitory effect of Gpp[NH]p on FK-stimulated AC was markedly decreased in pancreatic acinar membranes from ethanol-treated and ethanol withdrawal (2 weeks) rats, which suggests an abnormality at the G_i level in these membranes that would explain the decreased inhibition of AC by SS after ethanol ingestion. These latter findings are consistent with data obtained by Bauché et al. [36] who found that ethanol in vitro was able to abolish the AC inhibitory response to the adenosine R_i analog, N^6-phenylisopropyladenosine (N^6-PIA). In addition, we examined the functional activity of G_i by exploring the ability of Gpp[NH]p to inhibit binding of ^{125}I-Tyr^{11}-SS to pancreatic acinar membranes [4,5,7]. Gpp[NH]p was a much less potent inhibitor of ^{125}I-Tyr^{11}-SS binding to pancreatic acinar membranes in ethanol-treated and ethanol withdrawal (2 weeks) rats than in control rats. This finding suggests that there is a less functional G_i in the ethanol-treated rats and provides a mechanism for the decreased inhibition of FK-stimulated AC by SS in this condition.

The SSLI content as well as the binding parameters of SS receptors in the control rats were similar to those previously reported by others [3,4,28]. Within the past two years, at least five SS receptor subtypes have been cloned [37]. The pancreas appears to express only SSTR2 [38]. Since there is a lack of evidence for ethanol metabolism by the pancreas [39,40] and the inhibitory effects of ethanol on the SS receptor/effector system are reversible, it is possible that the inhibitory effects of ethanol on the SS mechanism of action are direct. It has been hypothesized that the action of ethanol is the result of ethanol-induced changes in the fluidity of membranes [41] and that these changes in the membrane's physical characteristics would influence the function of membrane-bound proteins such as receptors [42].

It is conceivable that the changes in SS receptor number in pancreatic acinar membranes after chronic ethanol consumption may be due to the derangement in receptor biosynthesis. Various studies have led to the conclusion that chronic ethanol consumption interferes with genetic code transcription and protein synthesis [43].

Ethanol administration did not affect either the number or the affinity of secretin receptors as compared to control values. These results, therefore, suggest that the change in SS binding is not a non-specific effect and are consistent with a study of Uhlemann et al. [44] who found no changes in the number of secretin receptors in pancreatic acini after ethanol treatment.

These results suggest that the attenuated inhibition of AC by SS in pancreatic acinar membranes from ethanol-treated and ethanol withdrawal rats may be caused by decreases in both G_i activity and in the number of SS receptors. Alternatively, an uncoupling of SS receptors from G_i and/or a decrease in the level of functional G_i may result in both a decrease in the apparent B_{max} for SS binding and in SS-mediated inhibition of AC.

In summary, we have demonstrated that the SS receptor/effector system in pancreatic acinar membranes is altered by chronic ethanol consumption and withdrawal (2 weeks). This impairment may explain, at least partly, the increase in pancreatic exocrine secretion observed during chronic ethanol ingestion.

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