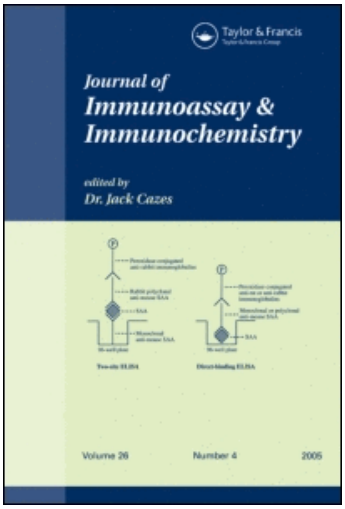

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THEOPHYLLINE INTERFERES WITH LIGAND BINDING IN RADIOIMMUNOASSAYS FOR SOMATOSTATIN

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ABSTRACT

Theophylline is often utilized as a secretagogue in studies on the in vitro release of somatostatin and other hormones. We report here on the effect of theophylline on ligand binding in somatostatin radioimmunoassays performed with three different antisera. For all three radioimmunoassays, tracer binding both in the presence and absence of unlabelled somatostatin was inhibited by the addition of theophylline to the RIA reaction mixture. This effect occurred at final assay concentrations of theophylline which may likely be encountered when assaying samples obtained in studies of theophylline-induced hormone release in vitro. The possible interfering effect of theophylline in radioimmunoassays should be considered whenever theophylline is used as a tool to study hormone release. (Key Words: Somatostatin, Theophylline, Radioimmunoassay)

INTRODUCTION

Theophylline, a known secretagogue for many hormones is widely utilized as a tool for the study of in vitro hormone release. In several recent in vitro studies of somatostatin secretion utilizing isolated pancreatic islets (1-4), the perfused pancreas (5, 6), and islet cell monolayers (7), theophylline was reported to act as a somatostatin secretagogue. Our experience

with theophylline and its effect on radioimmunoassays of somatostatin may therefore be of some importance.

We attempted to measure theophylline-induced somatostatin secretion from isolated rat islets incubated in vitro. Initial results appeared to indicate that the addition of theophylline to incubation medium effectively induced the release of somatostatin as determined by RIA. Theophylline increased somatostatin-like immunoreactivity (SLI) in the test incubation medium (theophylline present) vs. control medium (theophylline absent). However, when theophylline itself was added to incubation medium (in the absence of islets) the same increase in SLI was detected. Subsequently, we found that theophylline interfered with somatostatin tracer binding to three different antisera used for radioimmunoassay (RIA).

MATERIALS AND METHODS

Anti-somatostatin antisera 486 and AS-10 of established specificity (8, 9) were kindly provided by Drs. J. Gerich and J. Ensinck respectively. Anti-somatostatin antisera 1001 was kindly provided by Dr. T. Yamada. This antisera is directed toward the central portion of the somatostatin molecule, as determined by studies of somatostatin analogs and does not cross react with TRH, insulin, glucagon, vasoactive intestinal peptide, gastric inhibitory peptide, secretin, cholecystokinin, [Met]-or [Leu]-enkephalin, bombesin, substance P, ACTH, pancreatic polypeptide, or neurotensin from 10^{-15} to 10^{-9} M (personal communications with Dr. Yamada). Somatostatin radioimmunoassays were performed based upon the

method of Gerrich et. al. (8). Exact assay conditions, differing for each antisera, were established to result in useful standard curves for assay of biologic fluids.

For use as tracer with antibodies 486 and AS-10, [Tyr^{11}] somatostatin (Bachem) was iodinated and purified using Chloramine-T and Sephadex G-25(F) gel chromatography as described by Gerrich (8). For use with antibody (Ab) 1001, [Tyr^{1}] somatostatin was iodinated and purified similarly. Standards were prepared using synthetic cyclic somatostatin tetradecapeptide (Sigma). Assay buffer consisted of phosphate buffered saline at pH=7.4 for use with antibodies 486 and AS-10 and at pH=5.3 for use with Ab 1001. Tracer, standards, and Ab were all prepared in assay buffer to yield the final desired assay concentrations in a total assay volume of 600 microliters. Final Ab dilutions were 1/10,000 for Ab 486, 1/60,000 for Ab AS-10, and 1/175,000 for Ab 1001. Assays performed with Ab AS-10 and 1001 were incubated for 24 hours at 4°C with all reagents added at time 0. For assays using Ab 486, incubations were carried out at 4°C for 48 hours without tracer and then with tracer for an additional 24 hours. In all experiments, bound and free antigen were separated with dextran-coated charcoal.

For each antisera, experiments consisting of individual assay runs (N=4 for Ab 486 and Ab AS-10, N=5 for Ab 1001) were set up in duplicate and repeated at different times. The experiments were designed to determine the effect of theophylline (anhydrous, Sigma lot #99C-0065 or Kodak lot #AOA) on binding of labelled somatosta-

tin to Ab in the presence and absence of unlabelled somatostatin. A given assay run was set up to include: 1) the standard RIA curve, 2) standard RIA curves in the presence of theophylline, 1.66 mM and 0.833 mM (final assay concentrations) and 3) the effect of serial dilutions of theophylline starting with 1.66 mM (final assay concentration) on binding of labelled somatostatin to the Ab (in the absence of unlabelled somatostatin). The effect of theophylline on non-specific binding in the radioimmunoassays was also determined by setting up blank tubes (containing tracer with no Ab) with and without theophylline.

Statistical analysis was done using the paired t-test comparing points along the standard curves done in the presence of theophylline with the corresponding point along the curve determined (same assay) in the absence of theophylline. A one-tailed t-test was done looking for theophylline-induced interference with binding.

Theophylline, in concentrations used for these experiments, did not effect the pH of the assay buffer at either pH 7.4 or 5.3. The use of theophylline from both manufacturers, Kodak and Sigma, gave essentially the same results.

Inter- and intra-assay coefficients of variation respectively were 6.2% and 5.6% for assays using antisera 486, 5.8% and 2% for antisera AS-10, and 6.7% and 2.4% for antisera 1001. Control samples gave essentially the same results when assayed with the three different antibodies (coefficient of variation 3.8% for the means of controls run with the three antibodies).

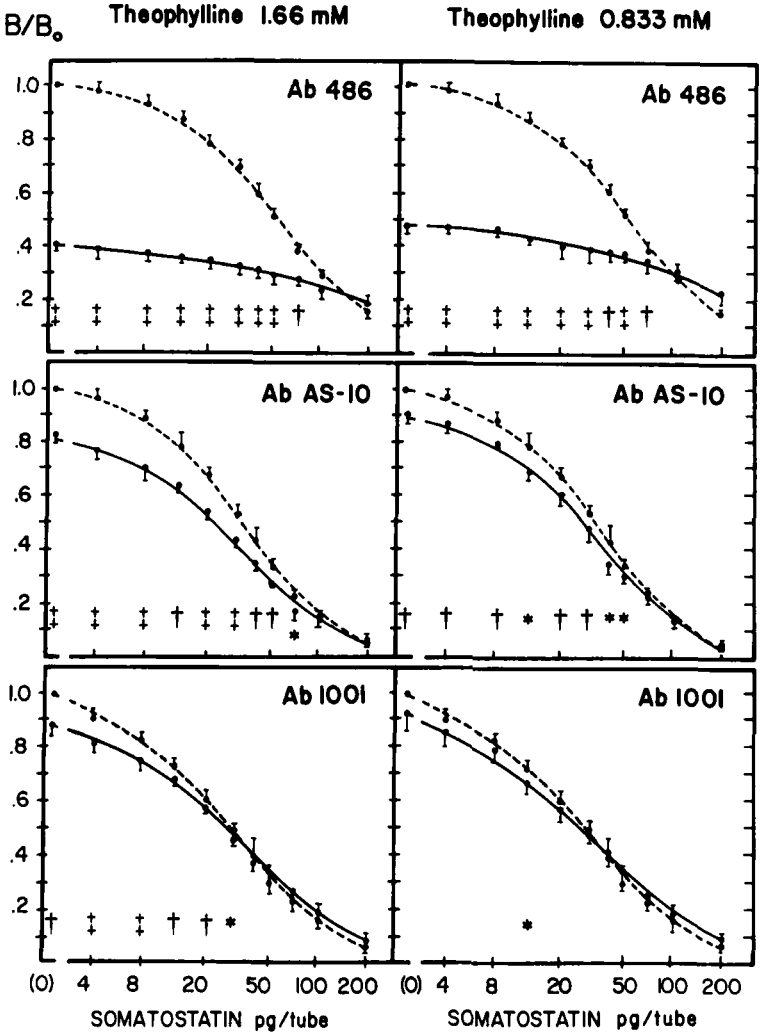

FIGURE 1. Standard somatostatin RIA curves run in the presence (——) of theophylline in final assay concentrations of 1.66 mM (left panel) and 0.833 mM (right panel) compared to the standard RIA curve run in the absence (---) of theophylline (for clarity, shown on both the right and left panels). Data are expressed as fraction of tracer binding (B_o) in the absence of both unlabelled somatostatin and theophylline versus log dose unlabelled somatostatin. The difference in the curves (theophylline present versus absent) reflects the effect of theophylline on the RIA standard curves for assays run with the Ab indicated. The curves are broken after dose = 0 on the abcissa since log zero is not expressible on the scale. N = 4 for antibodies 486 and AS-10. N = 5 for Ab 1001. B_o/Total = 20.9% for Ab 486, 49.3% for Ab AS-10 and 31.0% for Ab 1001. Half brackets = one SD. *p<.05 †p<.01 ‡p<.001 compared to corresponding point in absence of theophylline.

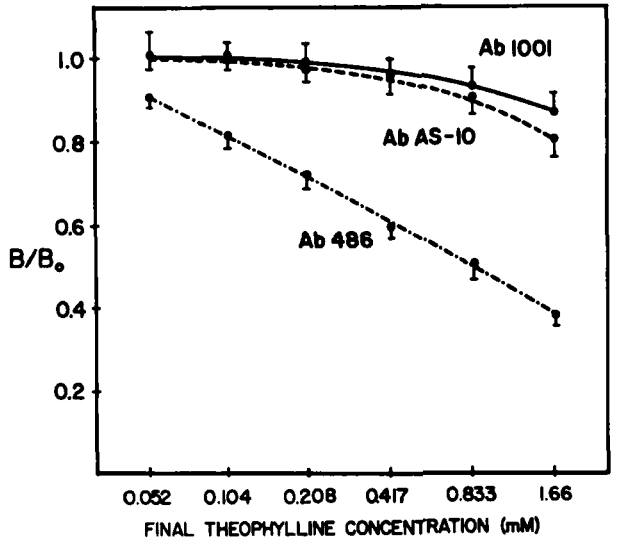

FIGURE 2. The effect of serial dilution of theophylline on binding of labelled somatostatin (no unlabelled somatostatin present) to the indicated Ab. Data are expressed as fraction of tracer binding (in the absence of both theophylline and cold somatostatin) to the Ab indicated versus log dose final assay theophylline concentration. The curves are not directly comparable since assay conditions were non-identical (see text) for the three antibodies studied. B_0/Total values for the three antibodies are indicated in Fig. 1 legend. N = 4 for Ab's 486 and AS-10. N = 5 for Ab 1001. Half brackets = one SD.

RESULTS

Theophylline (1.66 mM and 0.833 mM) decreased the binding of labelled somatostatin to each of the antibodies studied (Fig. 1). This effect varied with the Ab and associated assay conditions being most prominent in assays with Ab 486 and least prominent with Ab 1001. For antibodies 486 and AS-10, this effect was significant at both 1.66 and 0.833 mM theophylline for all points on the standard curves until the dose of added unlabelled ligand increased to a certain value at which time the difference between

the points on the curves became non-significant. This was also the case for Ab 1001 at a theophylline concentration of 1.66 mM. However, at a theophylline concentration of 0.833 mM the differences between the individual points on the curves lost significance.

Figure 2 illustrates the effect of theophylline in serial dilutions on tracer binding in the absence of unlabelled somatostatin. Again, the effect varied with the antibody studied. From these curves, it is evident that the antibodies "read" theophylline such that assay samples containing theophylline alone would appear to contain somatostatin.

There was no effect of theophylline on non-specific binding in the radioimmunoassays studied.

DISCUSSION

Theophylline concentrations used to stimulate somatostatin release *in vitro* have been in the range of 1.0 to 10 mM (1-7). The final assay concentration, of course, depends upon sample size relative to total assay volume. Our data indicate that theophylline, in final concentrations which may be encountered in studies of somatostatin release *in vitro*, is capable of interfering with binding in somatostatin radioimmunoassays. This effect varies with the particular RIA employed and is more significant if larger sample sizes are used relative to total assay volume.

Therapeutic theophylline concentrations used clinically generally range from 10 to 20 μg/ml or 0.056 to 0.111 mM. As shown in Figure 2, these concentrations are in the range which affect the RIA run with antisera 486. It is of concern, there-

fore, that when assaying plasma containing theophylline, some somatostatin radioimmunoassays could yield erroneous results.

The mechanism of action of theophylline as an interfering substance in the somatostatin radioimmunoassays is not clear from these studies. Chemically, theophylline is capable of binding to a wide variety of compounds (10) including protein (11). It is possible that theophylline, by binding to Ab at a site separate from the ligand immunorecognition site inhibits ligand binding non-competitively. If such were the case then one should be alert to a potential effect of theophylline on antibodies used for the immunoassay of ligands other than somatostatin.

Alternatively, theophylline could compete with somatostatin at the same Ab binding site. Kinetic study aimed at this question would be quite difficult, since the anti-somatostatin antisera we evaluated probably contain heterogeneous anti-somatostatin Ab populations or contain antibodies with more than one binding site. It should be pointed out that theophylline concentrations used in the experiments reported here represent a large molar excess over the amount of somatostatin tetradecapeptide required for displacement of tracer from antibody. This suggests that competitive effects, if present, are weak.

Although highly speculative, the prospect of theophylline competing with somatostatin for immunorecognition has implications as to the mechanism of action of somatostatin at the cellular level. For example, one could question whether the cellular effects of somatostatin could involve an enzyme system such as phosphodiesterase which is known to bind theophylline.

The difference in the magnitude of the effect of theophylline between the three radioimmunoassays studied could be due to a number of factors. Besides differences between the antisera themselves, the variables in assay conditions included the nature of tracer, pH, time of addition of tracer, and dilution of antisera. All of these factors are capable of affecting the degree to which an interfering substance affects a given RIA (12).

In summary, theophylline interferes with binding in somatostatin radioimmunoassays performed with three different antisera. In studies of theophylline-induced somatostatin secretion, this effect should be considered and controlled for in the RIA. A possible effect of theophylline on binding in other radioimmunoassays (for other ligands) may also have to be taken into account.

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