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Effect of Ethanol on Parathyroid Hormone and Calcitonin Secretion in Man (40311)

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Peng *et al.* (1, 2) showed that ethanol can induce hypocalcemia in dogs and in intact and parathyroidectomized rats, which could not be prevented by exogenous parathyroid hormone (PTH). Ramp *et al.* (3) observed ethanol-induced hypocalcemia in chickens. Subsequent studies in the rat from our laboratory (4) showed that ethanol caused a dose related hypocalcemia and an increase in PTH secretion which, however, was not sufficient to correct the hypocalcemia. The present study (a) evaluated the effect of ethanol on PTH and calcitonin (CT) secretion *in vivo* in normal man, and (b) evaluated the mode of ethanol effect on PTH secretion by studying its effect on bovine parathyroid tissue *in vitro*.

Materials and methods. Human studies. Normal male subjects aged 25–50 years, on normal diets and with no evidence of renal, calcium (Ca) metabolic or other endocrine abnormalities underwent the alcohol ingestion test. Informed consent was obtained from each subject to undergo this procedure, which had been approved by the Human Investigation Committee of this institution. The subjects were fasted for 12–15 hr before the procedure and were recumbent during the procedure. A scalp vein needle was placed in an antecubital vein and attached to an infusion set via a 3-way stopcock for the slow administration of normal saline (0.5 ml/min) and withdrawal of serial blood specimens. After a 30 min rest period, blood specimens were obtained for plasma immunoreactive (i) CT and for serum iPTH and Ca at −10 and −5 min for baseline values. The subject then drank ethanol (0.8 g/kg) in the form of 86 proof bourbon whiskey (one fourth of total dose at 0, 20, 40 and 60 min). Additional blood specimens were obtained at ½, 1, 1½, 2, 3, 4 and 4½ hr from the time ethanol ingestion was begun. A control group of five normal male subjects underwent a procedure which was similar except that ingestion of a volume of tap water approximating the volume of ethanol was substituted for the ingestion of ethanol.

A portion of each blood specimen was placed in a chilled heparinized tube, centrifuged in a refrigerated centrifuge and the plasma separated and frozen immediately for subsequent analysis of iCT. The other portion of the blood specimen was placed in a plain tube, allowed to clot for 1 hr, centrifuged, and the serum separated and frozen for subsequent iPTH and Ca determination.

Serum iPTH was determined by a method developed in this laboratory (5) using a guinea pig antibovine PTH antiserum, purified bovine PTH (Wilson Laboratories, lot 147865) for tracer, and dilutions of a pool of human parathyroid tissue culture medium for standards. This antiserum detects both the intact molecule and the amino terminal fragment of bovine iPTH, its molar affinity for bovine PTH 1-34 being approximately one half that for the intact PTH 1-84 molecule in the utilized portion of the standard curve. This antiserum has a high affinity for human and monkey iPTH, and detects dilutions of human serum and purified bovine PTH with superimposable displacement curves over a 60-fold dilution span. The normal mean value for human serum iPTH is 6.2 μl eq standard human parathyroid tissue culture medium/ml (μl eq/ml) with a normal range (mean ± 2 SD) of 3.8–8.6 μl eq/ml.

Plasma iCT was determined by a method developed in this laboratory (6, 7) using a goat antihuman synthetic CT antiserum and human synthetic CT (N.V. Organon, batch #SC 30) for standard and tracer. The normal mean value for human adult male plasma

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iCT is 218 pg/ml with a normal range (mean ± 2 SD) of 55–380 pg/ml.

Ethanol was added to serum and plasma specimens to a concentration of 1.6%, allowed to incubate at 4° for 2 hr and then assayed for iPTH and iCT respectively to determine whether ethanol may cause any degradation of these hormones or modification of the displacement of tracer, which would modify the assay-detected concentrations of iPTH or iCT.

Serum Ca concentration was determined by a modification of the method of Hill (8). The normal mean value is 9.2 mg/dl with a normal range of 8.2–10.2 mg/dl.

In vitro studies. Fresh bovine parathyroid tissue slices were incubated for 4 hr in Eagle Minimal Essential Medium with 10% calf serum by the technique previously described from this laboratory (9). The medium was completely aspirated and replaced by fresh medium hourly. During the first 2 hr the medium in all flasks contained 1.25 m*M* Ca (considered to approximate the ionized Ca concentration of normal plasma). The first hr of incubation was considered an equilibration period and this medium was discarded. The iPTH in the medium removed at the end of the next hr was considered to represent the control or zero-time baseline secretion of the tissue in that flask. The composition of the medium was then modified to contain either a high (3.0 m*M*) or a low (0.75 m*M*) Ca concentration or to contain either 0.05% or 0.3% ethanol, and incubation was continued for 2 additional hr. The iPTH concentration of each hourly medium sample was determined by radioimmunoassay as previously described (9), using purified bovine PTH for standard and tracer. The concentration of iPTH in pg/mg wet wt of parathyroid tissue in the zero-time baseline medium sample of each flask was designated as 100%. The iPTH concentration in the medium harvested at the end of each hr for the next 2 hr was then expressed as a percent of this zero-time baseline value for that flask (9). At least three control flasks containing 1.25 m*M* Ca during the entire incubation period were included with each group of incubation flasks to evaluate uniformity of secretion with time. The percent of zero-time baseline values obtained on hours 1 and 2 with the control flasks were then adjusted to 100%, and the data for each of the other flasks corrected to this adjusted baseline. Also, aliquots of media incubated without tissue were assayed to determine whether the ethanol had any nonspecific effects on the immunoassay results.

In all studies the mean and SE for each time period were calculated from the individual percent values for the time period for each subject (*in vivo* studies) or each flask (*in vitro* studies). Statistical tests of significance were carried out with Student's *t* test.

Results. Human studies. The mean baseline values (mean ± SE) for the six human subjects were: iPTH–6.6 ± 0.30 μl eq standard human parathyroid tissue culture medium/ml, iCT–269 ± 24.5 pg/ml, Ca– 9.4 ± 0.11 mg/dl. The direct addition of ethanol to serum or plasma caused no change in iPTH or iCT concentrations from those observed in the serum or plasma without addition of ethanol. As indicated in Fig. 1, infusion of normal saline and ingestion of water by normal subjects caused no change in iPTH, iCT or Ca. However, as shown in Fig. 2, ingestion of ethanol caused a significant ($P < 0.01$) increase in iPTH to 107.2 ± 2.11% of baseline by 30 min, at a time when only half of the ethanol had been ingested. The iPTH concentration continued to increase, reaching a peak value of 138.9 ± 4.44% of baseline ($P < 0.001$) at 2 hr, with gradual decrease thereafter to 106.0 ± 8.10% of baseline at 4½ hr. Plasma

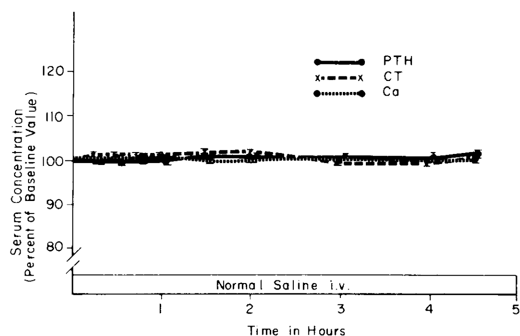

FIG. 1. Effect of iv infusion of normal saline and ingestion of tap water on serial serum iPTH, plasma iCT and serum Ca concentrations during a 4½-hr period in normal man. Values (mean ± SE) at each time period are expressed as percent of the baseline pre-ingestion values (designated as 100%). $N = 5$. Baseline values: iPTH – 6.4 ± 0.28 μl eq/ml, iCT – 240 ± 22.3 pg/ml, Ca – 9.3 ± 0.12 mg/dl.

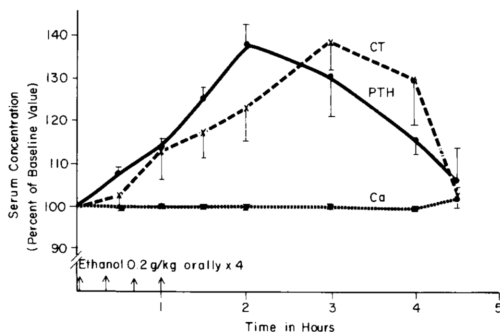

FIG. 2. Effect of ethanol ingestion on serial serum iPTH, plasma iCT and serum Ca concentrations during the following 4½ hr in normal man. Values (mean ± SE) at each time period are expressed as per cent of the baseline pre-ingestion values (designated as 100%). $N = 6$. Baseline values: iPTH – 6.6 ± 0.30 μl eq/ml, iCT – 269 ± 24.5 pg/ml, Ca – 9.4 ± 0.11 mg/dl.

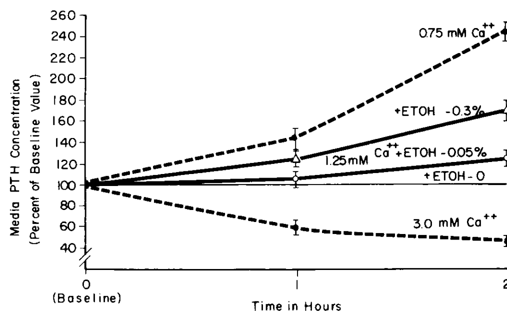

FIG. 3. Effect of Ca concentration and of concentrations of ethanol on hourly iPTH secretion *in vitro* by normal bovine parathyroid tissue. Medium prior to zero time contained 1.25 mM Ca and no ethanol in all flasks. When medium containing 1.25 mM Ca was continued during the entire incubation period (control flasks), the iPTH values (mean ± SE) were: 325 ± 13.8, 311 ± 22.4 and 319 ± 14.0 pg/mg wet wt parathyroid tissue at 0, 1 and 2 hr respectively. Each of these values was designated as 100% baseline for that hr. Each value is expressed as per cent of the zero time iPTH secretion for that flask, (corrected for the minimal variation of secretion in control flasks for that hr). Each point is the mean ± SE for six flasks.

iCT concentration increased more slowly, showing a significant ($P < 0.05$) rise to 115.0 ± 6.07% of baseline at 1½ hr, reaching a peak value of 137.8 ± 7.13% of baseline ($P < 0.001$) at 3 hr and then decreasing to 101.9 ± 1.90% of baseline at 4½ hr. Serum Ca did not significantly change at any time tested.

In vitro studies. Aliquots of medium (with or without added ethanol) which had been incubated without parathyroid tissue revealed no modification of the trace B/F ratio, indicating that neither the medium nor ethanol had any nonspecific effects on the immunoassay results. Changes in *in vitro* secretion of iPTH, related to medium changes in Ca ion concentration or to addition of ethanol, are portrayed in Fig. 3. Hourly iPTH secretion revealed only minimal variation when medium containing 1.25 mM Ca was used during the entire incubation period: (iPTH = 325 ± 13.8, 311 ± 22.4 and 319 ± 14.0 pg/mg wet wt of parathyroid tissue/hr at 0, 1 and 2 hr respectively). Each value was designated at 100% for that hr. Medium containing low (0.75 mM) Ca caused a significant ($P < 0.001$) increase in iPTH release to 142.5 ± 9.41% and 240.2 ± 8.10% of baseline at the first and second hr of incubation respectively. Medium containing high (3.0 mM) Ca caused a significant ($P < 0.001$) decrease to 57.3 ± 4.63% and 42.7 ± 4.23% of baseline at the first and second hr of incubation respectively. Addition of two concentrations of ethanol to 1.25 mM Ca medium caused increases in iPTH secretion. At a concentration of 0.05% ethanol, the increase to 105.1 ± 6.10% of baseline at 1 hr was not significantly different from baseline, but the iPTH increase to 122.1 ± 6.74% at 2 hr was significantly ($P < 0.02$) increased. The 0.3% concentration of ethanol caused increases in iPTH secretion to 124.1 ± 6.35% at 1 hr and to 166.3 ± 11.26% of baseline at 2 hr, both being significantly ($P < 0.02$ and $P < 0.001$ respectively) increased from baseline secretion.

Discussion. Our initial studies of the effect of ethanol on Ca metabolism in the rat (4) suggested that decrease in serum Ca was the primary event and the observed increase in serum iPTH was in response to the hypocalcemic effect of ethanol. However, this compensatory increase in iPTH did not prevent or fully correct the hypocalcemia. We proposed (through without supporting data) that ethanol may induce a decrease in bone resorption, leading to hypocalcemia and a relative skeletal resistance to the resorptive action of PTH. However, the studies of Peng *et al.* (2) suggest that decreased bone resorption does not occur and that there may be a shifting of Ca from extracellular fluid into tissues to explain the hypocalcemia. This ex-

planation is strengthened by the observations of Ramp *et al.* (3) that adding ethanol to the organ culture medium enhanced mineral accretion by embryonic chick bone.

The present study indicates that, in normal man, ethanol induces an increase in both serum iPTH and iCT without detectable change in plasma Ca. This observation could be explained by ethanol-induced decrease in bone resorption or increase in bone accretion, but without skeletal resistance to PTH. In this situation very minimal hypocalcemia would induce increased PTH secretion, with rapid bone resorption and restoration of serum Ca to normal, so that hypocalcemia was never detectable. However the *in vitro* observations indicate that a primary change in serum Ca is not the total explanation of the changes in serum iPTH. In this situation ethanol had a direct stimulatory effect on the parathyroid cell which was dose-related. It is therefore possible that ethanol has both an indirect (via induced hypocalcemia) and a direct effect on PTH secretion. The effect of ethanol on the C cell of the thyroid was not studied *in vitro*, but is inferred to also be direct, leading to increase in CT secretion. The simultaneous increase in PTH and CT secretion may at least partially explain the lack of changes in serum Ca in the present study.

Other investigators have reported that ethanol can stimulate CT secretion in patients with medullary carcinoma of the thyroid, and have proposed ethanol ingestion as a CT secretagogue (along with calcium infusion and pentagastrin injection) as a diagnostic test for this tumor (10–14). Initially, it could not be demonstrated that ethanol affected CT secretion in normal subjects (13), but a subsequent study, using a more sensitive assay method, demonstrated that some normal subjects do show a CT response to ethanol (14). In the present study using a larger dose, ethanol elicited a CT response in all six normal subjects tested. We are not aware of a previous report of the effect of ethanol on PTH secretion.

The lower media concentration (0.05%) of ethanol in the *in vitro* study is comparable to the average blood ethanol concentration achieved in social drinking situations, and the 0.3% media concentration of ethanol is comparable to the blood ethanol level achieved by severely intoxicated subjects (15). The doses of ethanol (0.8 g/kg) ingested by the human subjects in the present study rendered them only moderately intoxicated. Therefore ethanol, in amounts often ingested by social drinkers, increases both PTH and CT secretion, and therefore may modify Ca homeostasis.

Summary. Ingestion of 0.8 g/kg ethanol in 1 hr by normal man caused significant increases in both serum PTH and plasma CT concentrations, with peak values of 139% of baseline at 2 hr for PTH and of 138% at 3 hr for CT. Serum Ca did not change during the period of observation. Incubation of bovine parathyroid slices in 1.25 m*M* Ca Eagle media with 0.05% or 0.3% ethanol caused significant increases in PTH secretion to 122% and 166% of baseline respectively. Therefore: (1) *in vitro*, ethanol can be demonstrated to directly stimulate PTH secretion, (2) *in vivo*, ethanol ingestion induces an increase in PTH without detectable hypocalcemia, suggesting (a) prompt PTH secretion and action to compensate for a hypocalcemic effect of ethanol, so that actual hypocalcemia is not detectable, and/or (b) direct parathyroid stimulation. Though the exact mechanisms are unclear, the data indicate that ethanol, in amounts often ingested by social drinkers, increases both PTH and CT secretion, and therefore may modify Ca homeostasis.

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