Investigation of the effect of acepromazine on intravenous glucose tolerance tests in dogs

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Objective—To investigate the effects of administration of acepromazine on IV glucose tolerance tests (IVGTTs) in dogs.

Animals—8 male mixed-breed dogs.

Procedure—With a 1-week interval between tests, each dog underwent (in random order) an IVGTT with or without pretest administration of acepromazine maleate (0.1 mg/kg, SC, 30 minutes prior to the start of the IVGTT). Food was withheld from the dogs for 14 hours prior to each test. Blood samples were obtained at 20, 10, and 1 minute prior to and at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 minutes after administration of glucose.

Results—There were no significant differences in the baseline (ie, after food was withheld) plasma glucose, lactate, and insulin concentrations between dogs undergoing the IVGTT and acepromazine-IVGTT; however, lower baseline free fatty acid concentration was observed in acepromazine-treated dogs. Analysis of data via the application of Bergman's minimal model of glucose kinetics revealed no differences in insulin sensitivity, acute insulin response to glucose, disposition index, or glucose effectiveness between dogs treated or not treated with acepromazine before testing.

Conclusions and Clinical Relevance—Results indicated that in dogs undergoing IV glucose tolerance testing, pretest administration of small doses of acepromazine can be used as a means of chemical restraint without interfering with results of the glucose metabolism assessment. (*Am J Vet Res* 2004;65:1124–1127)

Acepromazine maleate is a phenothiazine derivative that binds to the dopamine receptors in the brain. The compound acts as a depressant of the CNS by affecting the basal ganglia, hypothalamus, limbic system, brainstem, and reticular activating system.[1] It causes sedation, muscular relaxation, and a reduction in spontaneous muscular activity; therefore, acepromazine is used as a tranquilizer and as a preanesthetic agent in dogs and other small animals. Because of its low toxicity and rapid action, chemical restraint of dogs with acepromazine is widely used in research settings and veterinary practice (eg, before physical examination, surgery, or the performance of various tests).[2,3] However, it is not known if administration of acepromazine interferes with results obtained during testing of glucose tolerance in dogs.

One of the tests used to assess carbohydrate metabolism and glucose tolerance is the **intravenous glucose tolerance test** (**IVGTT**). Performing an IVGTT is frequently coupled with calculation of various indices to describe glucose and insulin kinetics. A computer program has been developed that estimates indices of glucose-insulin dynamics using glucose and insulin data obtained from an IVGTT; it is based on Bergman's minimal model, which is the simplest model that could account for glucose and insulin kinetics.[4,5] Several indices are calculated from such an analysis of an IVGTT. The **insulin sensitivity index** (S_I) quantifies the capacity of insulin to promote glucose disposal. Insulin sensitivity is decreased in humans and other animals with type 2 diabetes, compared with that of healthy individuals. Moreover, insulin sensitivity is decreased before the onset of diabetes. Therefore, alterations in insulin sensitivity that are detected early provide an important research and clinical tool for monitoring alterations in glucose metabolism. Likewise, **glucose effectiveness** (S_G), which is the capacity of glucose to mediate its own disposal, can indicate perturbations of glucose metabolism independent of insulin action. Another key element of the glucose tolerance analysis is the **acute insulin response to glucose** (**AIRg**) that quantifies the adequacy of pancreatic insulin response to a glucose load and thereby provides information about the beta cell function. When glucose tolerance is maintained, decreased insulin sensitivity will be compensated by increased insulin secretion or action. This relationship is described by the disposition index (the product of SI and AIRg). The index is a convenient and accurate tool to describe the status of glucose tolerance and identify incipient impairment.[6-8]

It is not known if acepromazine interferes with glucose metabolism in a manner that would make it difficult to interpret the results of an IVGTT. The purpose of the study reported here was to investigate the effect of administration of acepromazine on results of IVGTTs in dogs.

Materials and Methods

Animals—Eight sexually intact male mixed-breed dogs (mean weight, 27.2 ± 1.1 kg) were included in the study. Sixteen experiments were performed with dogs in a conscious relaxed state. Food was withheld from the dogs for approximately 14 hours prior to each experiment; a meal was provided to the dogs at 9 AM on the day before the experiment, and any remaining food was removed at 5 PM. Dogs were housed under controlled kennel conditions (light, 12 hours; dark, 12 hours) in the University of Southern California Medical

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School Vivarium and fed once a day with a standard diet (26% protein, 15% fat, and 40% carbohydrate[a]). Dogs were used for experiments only if judged to be in good health, as determined by measurement of body temperature, assessment of Hct, regularity of food intake, and direct observation of behavior in the weeks prior to experiment, as well as on the day of the experiment. All surgical and experimental procedures were approved by the University of Southern California Institutional Animal Care and Use Committee.

Experimental procedures—Each dog underwent an IVGTT (without medication) and an IVGTT after administration of acepromazine (in random order) at a 1-week interval. The experimental protocol for the IVGTT and acepromazine-IVGTT was identical, except that in the latter procedure, acepromazine[b] (0.1 mg/kg) was injected, SC, 30 minutes before collection of the baseline samples.

The IVGTTs were performed as described.[8,9] Dogs were familiarized with the Pavlov sling at least 1 week before the experiments. At 7 AM on the day of the experiment (after food had been withheld), the dogs were brought in the laboratory and placed in the Pavlov sling. Each dog had two 19-gauge catheters[c] placed: 1 in a saphenous vein (for sample collection) and 1 in a cephalic vein (for infusion of glucose). Approximately 30 minutes after the placement of catheters, basal samples were obtained. Blood samples (3 mL) were drawn from the saphenous vein at 20, 10, and 1 minute prior to administration of glucose (at time 0). Baseline values (ie, after the withholding of food) were defined as the mean of the 3 basal samples (obtained at time –20, –10, and –1 minutes). At time 0, glucose (50% dextrose; 454 mg/mL) was administered IV via the cephalic vein at a dose of 0.3 g/kg. Additional blood samples were obtained at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 minutes, as described.[8]

Samples for determination of plasma glucose, lactate, **free fatty acid** (**FFA**), and insulin concentrations were collected into chilled tubes coated with lithium fluoride and heparin containing 50 µL EDTA (2% w/vol). All samples were stored on ice until centrifugation, after which plasma was separated into 2 aliquots; 1 aliquot was used immediately to measure glucose and lactate concentrations and then stored at –20°C for insulin measurement. To prevent triglyceride breakdown, the plasma samples for FFA assessment (second aliquot) were kept on ice and either immediately assayed or kept at –80°C until FFA determination.

Assays—Plasma glucose and lactate concentrations were determined online by use of an enzyme electrode autoanalyzer.[d] The instrument uses the glucose oxidase method and the lactate oxidase method with a redox electrode for analysis. Plasma insulin concentration was measured by use of a human insulin ELISA kit[e] that had been adapted in our laboratory for analysis of samples of plasma from dogs. The assay uses 2 monoclonal antibodies that bind to different epitopes on the insulin molecule and that do not bind to proinsulin. The ELISA cross-reacts well with canine insulin and has been previously validated in our laboratory.[10] Plasma FFA concentrations were determined by use of an enzymatic colorimetric assay[f] based on the acylation of coenzyme A.

Calculation of minimal model parameters—Values of S_I and S_G were calculated by the application of the minimal model of glucose kinetics to the time course of plasma glucose and insulin concentrations obtained from the IVGTT by use of computer software.[g] The value of AIRg was calculated as the **area under the curve** (**AUC**) of the plasma insulin concentrations above the mean baseline value from time 0 to 10 minutes. For each experiment, the disposition index was calculated as S_I multiplied by AIRg. Area under the curve was calculated as increase from zero (total AUC) or increase from basal values (AUC above baseline) by use of the trapezoidal rule. The AUC above baseline value from time 0 to 15 minutes of the IVGTT was designated $AUC_{0\text{-}15\ min}$.

Statistical analyses—Results are presented as mean values ± SE. To compare variables between dogs undergoing IVGTT with and without administration of acepromazine, the *t* test or Wilcoxon signed-rank test was used, depending on the normality of distribution. The time courses of the plasma glucose and insulin concentrations were analyzed by use of repeated measures ANOVA. For all analyses, differences were considered significant when $P < 0.05$. All analyses were performed with computer software.[h]

Results

Dogs had signs of sedation approximately 15 minutes after administration of acepromazine as assessed by subjective evaluation. Of the 8 dogs, 4 had moderate sedation and preferred to remain recumbent. The other 4 dogs had signs of slight sedation; each dog was able to stand but did not appear alert (signs of sedation included hanging of the head, drooping of the ears, and protrusion of the third eyelid).

The baseline values of plasma glucose, lactate, and insulin concentrations were not different in the presence or absence of acepromazine (**Table 1**). There was a significant ($P = 0.04$) suppression in basal plasma FFA release after acepromazine treatment; the baseline

Table 1—Mean ± SE baseline* plasma concentrations of glucose, lactate, insulin, and free fatty acid (FFA) in 8 dogs undergoing IV glucose tolerance tests (IVGTTs) with and without pretest administration of acepromazine maleate (0.1 mg/kg, SC).

	Test	
Variable	**IVGTT**	**Acepromazine-IVGTT**
Plasma glucose concentration (mg/dL)	91.9 ± 1.8	93.5 ± 2.6
Plasma lactate concentration (mg/dL)	5.7 ± 0.3	6.8 ± 0.7
Plasma insulin concentration (µU/mL)	6.1 ± 1.1	5.2 ± 0.8
Plasma FFA concentration (mM)	0.6 ± 0.2	0.4 ± 0.2†

*Baseline values (ie, after the withholding of food) were defined as the mean of the 3 basal samples obtained at 20, 10, and 1 minute prior to administration of glucose (at time 0). †Value significantly ($P < 0.05$) different from baseline value before IVGTT without administration of acepromazine.

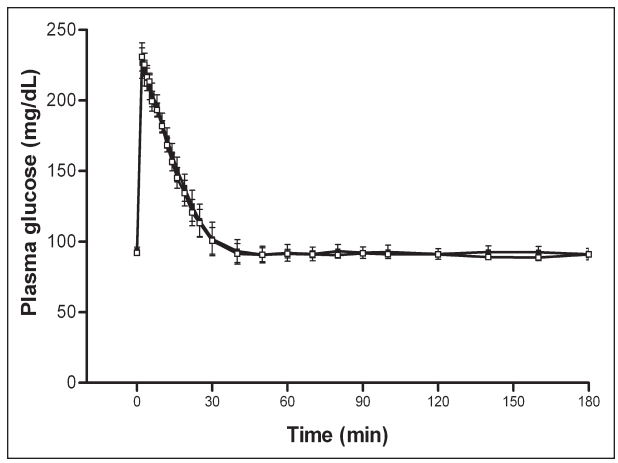

Figure 1—Plasma glucose concentration in 8 dogs undergoing IV glucose tolerance tests (IVGTTs) without (open squares) or with (closed squares) pretest administration of acepromazine maleate (0.1 mg/kg, SC, 30 minutes before administration of glucose bolus).

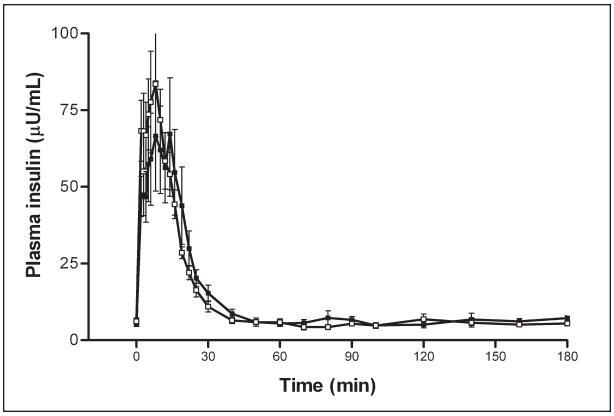

Figure 2—Plasma insulin concentration in 8 dogs undergoing IVGTTs without (open squares) or with (closed squares) pretest administration of acepromazine maleate (0.1 mg/kg, SC, 30 minutes before administration of glucose bolus).

Table 2—Mean ± SE values of insulin sensitivity (S_I), glucose effectiveness (S_G), acute insulin response to glucose (AIRg), and disposition index calculated from data obtained in 8 dogs undergoing IVGTTs with and without pretest administration of acepromazine maleate (0.1 mg/kg, SC).

	Test	
Variable	**IGVTT**	**Acepromazine-IVGTT**
S_I ($[\mu U/mL]^{-1} \times min^{-1}$)	6.6 ± 1.0	7.7 ± 2.3
S_G (min^{-1})	0.04 ± 0.01	0.03 ± 0.00
AIRg (μU/mL × min)	611 ± 102	458 ± 107
Disposition index ($S_I \times$ AIRg)	3,876 ± 864	3,209 ± 1,176

concentration was 0.4 ± 0.2mM without administration of acepromazine, compared with 0.6 ± 0.2mM after administration of acepromazine.

After glucose administration, plasma glucose concentration increased to a similar extent during the IVGTT and the acepromazine-IVGTT. At 2 minutes after glucose administration, plasma glucose concentration was 230.7 ± 10 mg/dL during the IVGTT and 226.4 ± 30.3 mg/dL during the acepromazine-IVGTT. From this peak value, plasma glucose concentration decreased during both tests over an almost identical time course, returning to approximately baseline values at the end of each test (**Figure 1**). In the IVGTT, the plasma glucose concentration at 180 minutes was 89.6 ± 2.1 mg/dL (baseline value, 91.9 ± 1.8 mg/dL); in the acepromazine-IVGTT, the plasma glucose concentration at 180 minutes was 91.0 ± 11.4 (baseline value, 93.5 ± 7.2 mg/dL).

Administration of acepromazine did not significantly affect the plasma insulin concentration profile obtained during the IVGTT (**Figure 2**). Plasma insulin concentration increased to a mean peak value of 83.5 ± 17.1 μU/mL during the IVGTT and 66.6 ± 18.0 μU/mL during the acepromazine-IVGTT. There was no significant difference in the total insulin AUC, insulin AUC above baseline value, or insulin $AUC_{0\text{-}15\ min}$ between the IVGTT and the acepromazine-IVGTT experiments.

By application of the minimal model, S_I, AIRg, S_G, and disposition index were calculated. There were no significant differences in these parameters between IVGTT and the acepromazine-IVGTT (**Table 2**).

Discussion

Chemical restraint of dogs is sometimes necessary to perform glucose tolerance tests, but drugs used for this purpose may interfere with aspects of carbohydrate metabolism. Barbiturates are known to induce hyperglycemia and produce disturbances of glucose and FFA metabolism.[11-14] Benzodiazepines and xylazine have hyperglycemic effects as well.[15,16] Hyperglycemia can be induced in mice[17] and rats[18] by administration of chlorpromazine, which is a phenothiazine that is structurally related to acepromazine and used as an antipsychotic agent. In humans, prolonged treatment with low doses of chlorpromazine (75 mg/day for 7 days) did not modify glucose tolerance and glucose-stimulated pancreatic response, but acute doses (50 mg administered within 60 minutes) induced slight hyperglycemia.[19]

Less information is available about the effect of acepromazine on glucose tolerance. In cats, acetylpromazine (2.2 mg/kg, IM) produced significantly higher plasma glucose values during an IVGTT, compared with plasma glucose values in an IVGTT performed in the same animals but without the medication.[20] To our knowledge, the effect of administration of acepromazine on glucose tolerance in dogs has not been previously investigated. In the dogs of this report, acepromazine maleate administered at a dose of 0.1 mg/kg induced sedation but did not alter the baseline (ie, after food was withheld) plasma concentrations of glucose, insulin, or lactate (ie, values at baseline during IVGTT with and without acepromazine were not significantly different).

After IV administration of the glucose challenge during the IVGTT and the acepromazine-IVGTT in our study, the plasma glucose concentration profiles were almost identical. Although the mean insulin concentration profile appeared to have a smaller and delayed peak during the acepromazine-IVGTT, compared with that detected during the IVGTT, the clinical relevance of this finding is questionable because there was no significant difference between the 2 curves (repeated measures ANOVA, $P = 0.227$). There was also no significant difference in the total insulin AUC, insulin AUC above baseline, or insulin $AUC_{0\text{-}15\ min}$ between the 2 test conditions.

Our data also indicated that acepromazine had no effect on values of S_I, AIRg, and disposition index as calculated via minimal model analysis of the frequently sampled IVGTTs. Intravenous glucose tolerance testing coupled with the minimal model analysis is widely used to measure S_I because it is minimally invasive, relatively easy to perform, and inexpensive. Although the gold standard for assessment of S_I is the euglycemic hyperinsulinemic clamp (a model-independent method), the equivalence of the S_I values obtained via these 2 methods has been determined.[21]

An interesting finding of our study was the acepromazine-induced decrease in baseline (ie, after food was withheld) plasma FFA concentration, compared with findings during the IVGTT. Although no data are available regarding the effect of acepromazine on lipolysis, other phenothiazines such as chlorpromazine have been reported to increase serum FFA concentration in rats[22] and release FFA from subcutaneous adi-

pose tissue in dogs,[23] probably as a result of α-adrenergic receptor antagonist properties. However, phenothiazines affect the CNS and exert autonomic and endocrine effects through blocking other receptors including dopamine, muscarinic, H_1 histaminic, and serotonin receptors.[24] Recently, chlorpromazine has been identified as a noncompetitive inhibitor of the nicotinic acetylcholine receptor.[25] It is possible that acepromazine exerts an antinicotinic antagonist effect predominantly, resulting in suppression of lipolysis. Regardless, the results of the study reported here indicated that the administration of small doses of acepromazine to dogs does not influence the basal or glucose-stimulated parameters of glucose metabolism; therefore, it appears that acepromazine can be used as a means of chemical restraint prior to performing glucose tolerance tests in dogs.

[a]Prolab canine diet, PMI Nutrition International, Brentwood, Mo.
[b]Acepromazine maleate, 10 mg/mL, Boehringer Ingelheim, St Joseph, Mo.
[c]BD Intracath, Becton-Dickinson, Sandy, Utah.
[d]YSI 2300 autoanalyzer, Yellow Springs Instruments, Yellow Springs, Ohio.
[e]EZHI-14K, Linco Research Inc, St Charles, Mo.
[f]NEFA C kit, Wako Chemicals, Neuss, Germany.
[g]MINMOD Millennium software, version 5.8, 2002, BeBoS Assoc, Pasadena, CA.
[h]Sigmastat 3.0, SPSS 2003 software, SPSS Inc, Chicago, Ill.

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