ABSORPTION KINETICS OF REGULAR AND ISOPHANE (NPH) INSULIN IN THE NORMAL DOG

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ABSTRACT

Absorption kinetics of regular and isophane (NPH) insulins were evaluated in seven normal fasted dogs by measuring serial serum concentrations of insulin and glucose following the subcutaneous administration of regular and NPH insulins. These results were compared to serum insulin values determined after injecting similar doses of regular insulin intravenously. Regular insulin was better absorbed than NPH insulin (mean bioavailability index 64.6% vs. 41.1%, $P<.01$) resulting in a significantly greater maximal increase in mean circulating insulin concentrations above baseline values (362.2 μU/ml vs. 147.8 μU/ml, $P<.05$). The time interval between insulin injection and return of serum insulin values to basal concentrations was also significantly shorter for regular than for NPH insulin (4.9 hr vs. 8.6 hr, $P<.05$). However, there were no significant differences between regular and NPH insulins in time to reach peak serum insulin concentrations, maximal reduction in serum glucose concentrations, or time of lowest circulating glucose levels. The results of this study support previously accepted values for time-action characteristics of regular insulin, but suggest that NPH insulin may have an earlier peak and shorter duration of action than has previously been proposed in the dog.

INTRODUCTION

Although insulin treatment has been used in the management of canine diabetes mellitus for many years, little information concerning the absorption kinetics of insulin preparations commonly used in the dog is available. Traditionally, a single daily injection of an intermediate-acting insulin (eg. NPH or lente insulin) has been recommended for control of hyperglycemia in uncomplicated canine diabetes mellitus (1-3). More recently, however, a number of reports have suggested that this regime may fail to adequately maintain glucose homeostasis in a substantial number of dogs with diabetes mellitus (4-10). Following subcutaneous injection of insulin, many diabetic dogs develop their lowest blood glucose concentrations well in advance of the expected timing for peak action of the intermediate-acting insulins (4-7). In addition, the duration of insulin action also appears to be shorter than the expected 18 to 24 hours in many dogs with diabetes mellitus (4-10). These observations suggest that the onset, peak, and duration of action for the various insulin preparations may differ markedly from what has previously been proposed for the dog (1-3).

In this study, we evaluated the absorption kinetics of two commonly used insulin preparations by measuring serial serum concentrations of insulin and glucose following the subcutaneous administration of NPH or regular insulins

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to normal fasted dogs. These results were compared to the serum insulin values determined after injecting similar doses of regular insulin intravenously.

MATERIALS AND METHODS

Seven adult mix-breed dogs (4 females and 3 males) weighing 7.3 to 21.4 kg (mean ± SEM = 15.9 ± 1.8 kg) were used in this study. All dogs were judged to be healthy based on physical examination and results of a complete blood count and serum biochemical profile (SMA 12/60 AutoAnalyzer, Technicon Instruments Corp., Tarrytown, NY). The dogs were fed a commercially prepared dog food (Science Diet, Hill's Pet Products Inc., Topeka, KS) once daily, were allowed free access to water, and were housed in individual cages.

After a 24-hour fast, a purified pork insulin preparation was administered to each dog using a dose of 0.5 U/kg either intravenously (Regular Ilentin II injection, Eli Lilly and Co., Indianapolis, IN) or subcutaneously (Regular Ilentin II injection and NPH Ilentin II suspension, Eli Lilly and Co.) on three separate occasions. The studies were performed in random order at 2- to 4-day intervals; all three testing procedures were completed within a 10-day period. For the studies with regular insulin, blood was collected for determination of insulin and glucose concentrations before (-30 and 0 minutes) and 15, 30, 45, 60, 120, 180, 240, 300 and 360 minutes after the injection; additional samples were obtained at 5 and 10 minutes post-injection when the insulin was injected intravenously. For the NPH insulin studies, blood was collected for insulin and glucose determinations before (-30 and 0 minutes) and 0.5, 1.0, 1.5, 2, 4, 6, 8, 10, 12, 14, 16, 20 and 24 hours after administration. All subcutaneous insulin injections were given within a shaved 3 cm^2 area in the right upper thoracic wall.

For all glucose and insulin determinations, blood was centrifuged within 30 minutes of collection and the serum stored at -70C until assayed. Glucose concentrations were measured by the glucose-oxidase method on a Beckman glucose analyzer. Serum insulin was measured using a commercial radioimmunoassay kit (Micromedic Systems, Inc., Horsham, PA). Assay of serial dilutions of a canine serum pool with an insulin concentration of approximately 100 μU/ml resulted in inhibition curves with slopes parallel with the standard curve. Accuracy was determined by adding varying quantities of purified porcine insulin to a canine serum pool containing an undetectable concentration of insulin. Linear regression analysis of the resulting data (x = amount of insulin added: y = amount of insulin measured) gave the equation $0.93x + 2.23$, with a correlation coefficient of 0.99. The sensitivity of the insulin assay was 3.1 μU/ml; intra- and interassay coefficients of variation were 9.0% and 9.5%, respectively. To avoid between-assay variation in individual dogs, each dog had all samples from all three study days run in the same assay.

For the studies of regular and NPH insulins injected subcutaneously, the following parameters were analyzed: 1) maximum increase in serum insulin concentration over mean baseline value; 2) time interval between the insulin injection and the point at which serum insulin reached peak concentration; 3) time interval between the insulin injection and the return of serum insulin value to basal concentration; 4) maximum reduction in serum glucose concentration; 5) time interval between insulin administration and the lowest serum glucose value; 6) the bioavailability index, calculated by dividing the area under the serum insulin curve for the regular or NPH dose by the area under the serum insulin curve for the intravenous regular dose. All insulin

responses above the mean of the two baseline concentrations were regarded as exogenous insulin, regardless of the concomitant serum glucose concentration. The basis for this assumption is that both hypoglycemia and exogenous hyperinsulinemia suppress endogenous insulin secretion in the normal, fasted state (11). In addition, reactive hyperglycemia, when it occurs, is associated with increased circulating concentrations of epinephrine which also reduces endogenous insulin secretion (12,13).

All results are given as the mean ± SEM. Statistical analyses were performed using Student's paired and unpaired t tests; a P value of .05 or less was considered significant. The areas under the serum insulin curves were calculated using the trapezoidal rule (14). The elimination half-life of intravenous regular insulin was calculated by the least squares analysis from the decline of serum insulin concentrations between 15 and 180 minutes after administration (14).

RESULTS

Following intravenous administration of regular insulin, the mean serum insulin concentration peaked at 5 minutes (3710 ± 543 μU/ml) and returned to basal levels at 3 hours post-injection, with a mean elimination half-life of 18.4 ± 0.7 minutes (Figure 1A, Table 1). Mean serum glucose concentrations decreased significantly by 5 minutes and reached a nadir of 29 ± 3.1 mg/dl at 15 minutes post-injection. In individual dogs, the maximum reduction of serum glucose concentrations from basal values ranged from 77 to 87 mg/dl, whereas the time interval between insulin administration and the point at which lowest serum glucose levels were reached ranged from 10 to 45 minutes (Table 1).

After the subcutaneous injection of regular insulin, the mean serum insulin concentration increased significantly by 15 minutes and reached a peak value (312.6± 47.1 μU/ml) at 45 minutes post-injection (Figure 2). After a plateau of mean circulating insulin levels of approximately 300 μU/ml between 30 and 60 minutes, serum insulin concentrations fell and were statistically indistinguishable from basal concentrations at 4 hours (Figure 2). In individual dogs, the maximal increases in serum insulin concentrations over baseline values ranged from 187.5 to 555.4 μU/ml, the time interval between the insulin injection and the point at which circulating insulin reached peak concentrations ranged from 30 to 60 minutes, the time at which serum insulin returned to basal concentrations ranged from 4 to 6 hours, and the bioavailability index ranged from 61% to 73% (Table 2).

Mean serum glucose concentration decreased significantly by 15 minutes after the subcutaneous regular insulin injection, reached a nadir of 35.3 to 37.9 mg/dl at 60 to 120 minutes, and rose to a level indistinguishable from

TABLE 1. SERUM INSULIN AND GLUCOSE RESPONSES FOLLOWING INTRAVENOUS INJECTION OF REGULAR INSULIN IN NORMAL DOGS

Dog No.	Elimination t ½ (min)	Time insulin back to baseline (hr)	Maximum reduction of glucose (mg/dl)	Time of maximal glucose reduction (min)
1	17.3	3	87	30
2	18.4	3	81	30
3	20.2	3	78	30
4	18.7	3	78	45
5	14.9	3	78	15
6	18.6	3	77	10
7	20.4	3	87	15
Mean	18.4	3	80.9	22.9
±SEM	±0.7	±0.0	± 1.7	± 4.7

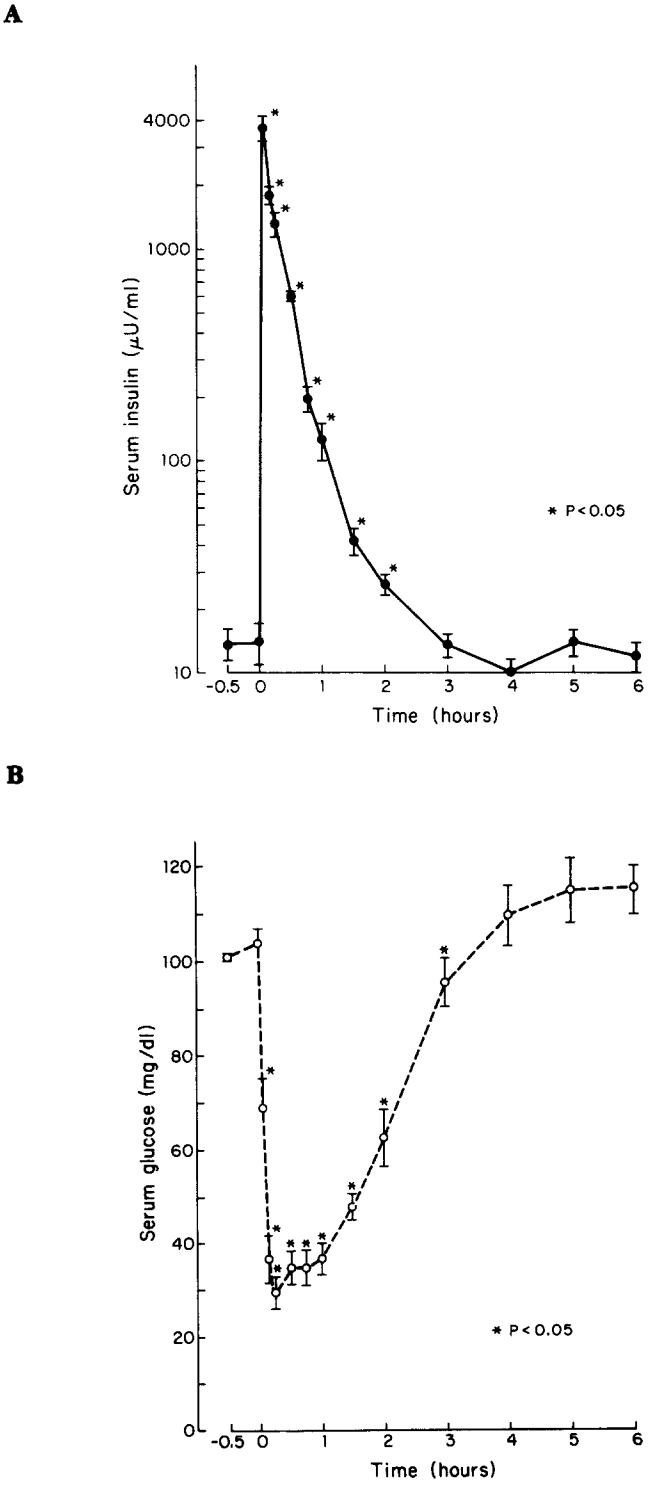

Fig. 1. Mean serum insulin (A) and glucose (B) concentrations in 7 dogs after intravenous administration of regular insulin.

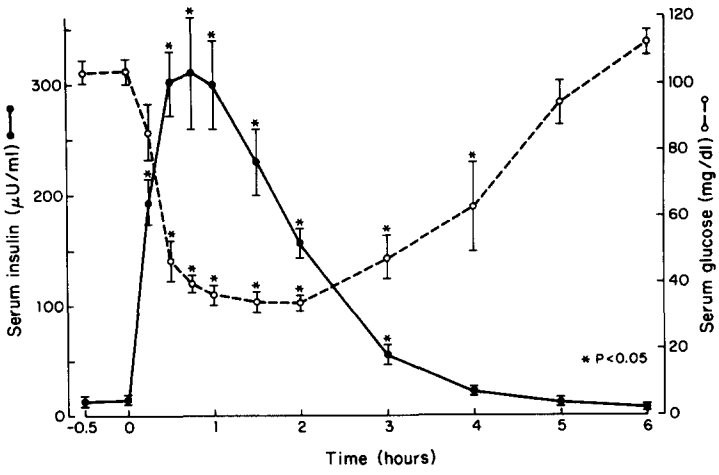

Fig. 2. Mean serum insulin and glucose concentrations in 7 dogs after subcutaneous administration of regular insulin.

basal concentrations at 5 hours post-injection (Figure 2). In individual dogs, the maximal reduction of serum glucose concentrations from basal values ranged from 64 to 94 mg/dl, whereas the time of the serum glucose nadir ranged from 45 to 120 minutes (Table 2).

Following the subcutaneous injection of NPH insulin, the mean serum insulin concentration rose significantly and reached a peak value (154.0 ± 56.7 μU/ml) at 30 minutes (Figure 3). Mean circulating insulin concentrations declined gradually and were not statistically different from basal values at 6, 8, 10, 12, 14, 16 or 24 hours post-injection. In individual dogs, the maximal increases in serum insulin concentrations over basal levels ranged from 27.7 to 329.1 μU/ml, the time at which circulating insulin reached peak concentrations ranged from 30 to 120 minutes, the time when serum insulin returned to basal concentrations ranged from 4 to 12 hours, and the bioavailability index ranged from 19% to 72% (Table 2).

Mean serum glucose concentration decreased significantly by 30 minutes after subcutaneous NPH insulin administration, reached a nadir of 45.9 to 46.7 mg/dl at 60 to 120 minutes, and rose to a level not significantly different from basal concentrations at 6 hours post-injection (Figure 3). In individual dogs, the maximal reduction of serum glucose concentrations from baseline values ranged from 48 to 87 mg/dl; time of the serum glucose nadir ranged from 30 to 240 minutes (Table 3).

TABLE 2. SERUM INSULIN AND GLUCOSE RESPONSES FOLLOWING SUBCUTANEOUS INJECTION OF REGULAR INSULIN TO NORMAL DOGS

Dog No.	Maximum insulin increase (μU/ml)	Time of Maximum insulin increase (min)	Time insulin back to baseline (hr)	Bioavail-ability index (%)	Maximum glucose reduction (mg/dl)	Time of maximum glucose reduction (min)
1	341.7	30	6	62	74	60
2	280.1	30	5	63	70	120
3	342.3	60	5	65	72	90
4	273.9	60	5	62	68	120
5	187.5	30	4	73	75	120
6	302.8	45	4	66	64	90
7	555.4	30	5	61	94	45
Mean	326.2	40.7	4.9	64.6	73.9	92.1
±SEM	±42.9	±5.4	± .03	±1.6	±3.6	±11.5

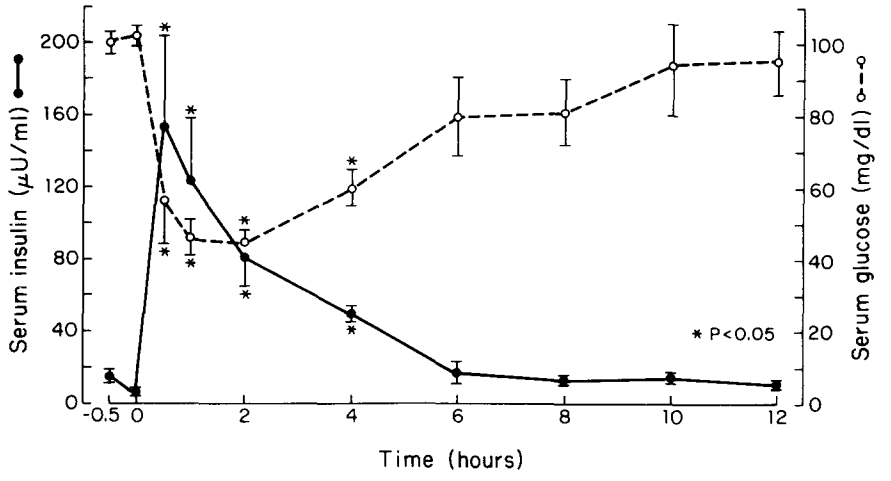

Fig. 3. Mean serum insulin and glucose concentrations in 7 dogs after subcutaneous administration of NPH insulin.

Compared to NPH insulin, regular insulin was better absorbed (mean bioavailability index 64.6% vs 41.1%, P<.01), resulting in a significantly greater maximal increase in mean circulating insulin concentrations above baseline values (326.2 μU/ml vs 147.8 μU/ml, P<.05). The time interval between insulin injection and return of serum insulin values to basal concentrations was also significantly shorter for regular than for NPH insulin (4.9 hours vs 8.6 hours, P<.05). There were no significant differences between regular and NPH insulin, however, in time to reach peak serum insulin concentrations, maximal reduction in serum glucose concentrations, or time of lowest circulating glucose levels (Tables 2 and 3).

DISCUSSION

The results of this study demonstrate that the characteristics of regular insulin after subcutaneous or intravenous administration to dogs are similar to previously suggested values for the dog and reports in man (1,2,3,15,16). Our data on NPH insulin, however, suggest that the properties of this insulin preparation are markedly different from what has been proposed for the dog and results of human studies (1,2,15,16). In the dogs of this report, NPH insulin was more rapidly absorbed, and produced earlier peak serum insulin

TABLE 3. SERUM INSULIN AND GLUCOSE RESPONSES FOLLOWING SUBCUTANEOUS INJECTION OF NPH INSULIN TO NORMAL DOGS

Dog No.	Maximum insulin increase (μU/ml)	Time of maximum insulin increase (min)	Time insulin back to baseline (hr)	Bioavailability index (%)	Maximum glucose reduction (mg/dl)	Time of maximum glucose reduction (min)
1	86.5	120	10	28	55	120
2	64.3	60	12	32	53	240
3	329.1	30	4	72	87	30
4	286.6	30	8	61	78	30
5	27.7	60	4	19	48	60
6	171.1	30	12	39	57	240
7	107.4	120	10	45	51	120
Mean	153.2	64.3	8.6	42.3	61.3	120.0
±SEM	± 43.4	±15.2	±1.3	±7.1	±5.7	±33.9

concentrations which returned to basal values much sooner than in comparable studies of absorption kinetics of NPH insulin in man (15,16).

The rapid absorption of NPH insulin with subsequent early peak serum insulin concentrations may be related to the use of a purified pork insulin preparation rather than a beef-pork product (5). In this study, purified pork insulins were chosen over beef-pork insulin preparations to prevent formation of insulin antibodies which can interfere with the direct radioimmunoassay of insulin (17). It has been suggested that insulin antibodies act as a buffer to prolong the effects of insulin by decreasing its immediate availability and prolonging its biologic half-life (16,18). Such antibodies have been found consistently in dogs receiving beef-pork insulin preparations, but have not been detected in dogs treated chronically with purified pork insulin (19). The differences in antigenicity beween beef-pork and purified pork insulin preparations may be explained by the fact that the latter has the same amino acid sequence as dog insulin (5,19), whereas beef insulin differs from dog insulin by two amino acids. Recently, however, the suggestion that insulin antibodies prolong the duration of insulin action has been questioned since no significant differences were found in insulin pharmacokinetics between antibody-positive and antibody-negative human patients with diabetes mellitus (20). Further studies are needed to determine the effects of insulin antibodies on insulin pharmacokinetics in the dog.

The onset of insulin action, as reflected by a significant decrease in pretreatment serum glucose concentrations, correlated with the increase in serum insulin concentrations and occurred shortly after the administration of regular or NPH insulin to the dogs of this report. We could not accurately determine the duration of action of regular or NPH insulin, however, because injection of either insulin preparation at the doses used in this study produced hypoglycemia, which would be expected to activate counterregulatory mechanisms and raise blood glucose concentrations despite persistence of insulin action. Therefore, calculation of the time for serum glucose to return to basal concentrations following insulin administration could greatly underestimate the duration of insulin action in our studies. Nevertheless, the duration of action of NPH insulin in most dogs is probably shorter than the proposed 18 to 24 hours (1-3), since it is unlikely that the duration of insulin action would persist for up to 12 hours after return of circulating insulin concentrations to low basal values. If the pharmacokinetics of insulin in the diabetic dog are comparable to this study, most dogs with diabetes mellitus may need twice daily injections of intermediate-acting insulin to adequately control the diabetic state.

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