Comparative Study of Immunoactivity and Bioactivity of Sodium Insulin

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Abstract □ The bioactivity of equal doses (by radioimmunoassay) of regular insulin and sodium insulin were measured in six preconditioned female mongrel hound dogs by a previously described bioassay. The hypoglycemic nadir of blood glucose induced by intravenous injection of the drugs was compared with the nadirs induced by a known amount of the international standard insulin. The level of blood glucose was monitored instantaneously and continuously using a nonthrombogenic continuous glucose-monitoring system. The hypoglycemic effect of subcutaneously injected sodium insulin was compared with the effect of subcutaneously injected regular insulin. The two drugs were injected at 1–2-week intervals to seven dogs. While the bioactivity of intravenously administered sodium insulin was equal to the bioactivity of intravenously administered *regular insulin*, the hypoglycemic effect of subcutaneously injected sodium insulin differed from the effect of subcutaneously injected regular insulin. The blood glucose nadir after subcutaneous injection of regular insulin occurred 101 ± 21 min after the injection. The nadir after subcutaneous injection of sodium insulin occurred 163 ± 33 min after the injection.

Keyphrases □ Sodium insulin—comparative study of immunoactivity and bioactivity □ Immunoactivity–comparative study of bioactivity of sodium insulin □ Bioactivity—comparative study of immunoactivity of sodium insulin

Sodium insulin is a new semisynthetic dosage form of insulin. Sodium insulin, like insulin, can be measured by an immunoassay, since both contain the same immunospecific binding site to the antibodies to insulin. However, equal doses of the two types of insulin, as measured by the immunoassay, are not necessarily equal in their biological activity. Also, equal biological activity after intravenous administration does not necessarily mean that the two types of insulin are of equal biological activity when injected subcutaneously.

An improved bioassay for insulin (1) was reported and is based on a previously described nonthrombogenic continuous glucose-monitoring device (2, 3). The bioassay was used to compare the intravenous biological activity of equal doses of sodium insulin and insulin standard (USP reference standard) measured by immunoassay. The hypoglycemic effect of subcutaneously administered sodium insulin was also compared with the effect of an equal dose of regular insulin.

EXPERIMENTAL

Immunoassay of Sodium Insulin—The immunoactivity of sodium insulin was measured six times, using a previously described immunoassay (4). The average of the six immunoassays was used to prepare the solution of sodium insulin used in the bioassay.

Bioassay of Sodium Insulin—*Instrument and Dogs*—Six preconditioned female mongrel hound dogs (16–17 kg) were used. The dogs were fasted overnight and anesthetized in the morning using sodium pentobarbital. Continuous glucose monitoring was initiated using a previously described nonthrombogenic system (2, 3). The instrument consisted of systems for nonthrombogenic blood withdrawal and for blood glucose measurement. The blood withdrawal system included a disposable sterile intravenous needle and tubing, coated with a nonthrombogenic substance connected to a peristaltic pump. The glucose-measuring system used a glucose oxidase sensor generating a continuous record of the level of blood glucose.

Priming Procedure—The bioassay was preceded by a priming procedure consisting of an intravenous administration of insulin followed by glucose.

The blood level of glucose was monitored for at least 30 min in order to establish the baseline level of glucose. Each dog was then given an injection of 0.4 U iv of USP reference standard insulin prepared in accordance with the USP procedure (5). The level of blood glucose fell rapidly reaching the initial priming nadir (N_0) within 30–35 min. After recording N_0, the level of glucose was raised by intravenous administration of 15–20 ml of a 25% solution of glucose. An additional 10 ml of glucose solution was given when necessary, until the blood glucose level stabilized within 10% of the baseline (~45–55 min).

Bioassay Procedure—After ascertaining maintenance of the glucose level at a consistent level for 10 min, the first dose of the USP standard insulin (0.4 U) was administered intravenously in one rapid bolus. The first nadir (N_1) was recorded by the glucose monitor within 35 min after the injection of insulin. As soon as an upturn in the level of blood glucose was established, the level of blood glucose was raised using a 25% intravenous glucose solution administered in 3 to 4 increments as previously described.

After ascertaining the reestablishment of a constant glucose level within 10% of baseline, the procedure was repeated twice: using 0.8 U of USP standard insulin, recording the second nadir (N_2) of blood glucose, and subsequently, using 0.6 U of sodium insulin as measured by immunoassay (4) and measuring the third nadir (N_3).

The bioactivity of the assayed sodium insulin was calculated using a least-squares linear regression of the logarithm of the two standard doses and the three nadirs.

Hypoglycemic Effect of Subcutaneous Sodium Insulin and Regular Insulin—Seven preconditioned female mongrel hound dogs (16–17 kg) were fasted and anesthetized as described previously. A nonthrombogenic catheter was inserted in one of the major veins of the front leg. The catheter was connected to the continuous glucose-monitoring device (2, 3). After a steady glucose level was observed for 10 min, 1 U of sodium insulin dissolved in sterile insulin diluent USP was injected

Table I—Six Repeated Bioassays of 0.6 U Sodium Insulin

Assay No.	Nadirs of Blood Glucose Levels, mg/dl: N_1 (0.4 U of Reference Standard)	N_2 (0.8 U of Reference Standard)	N_3 (0.6 U of Sodium Insulin)	Bioactivity of 0.6 U of Sodium Insulin
(1)	59	25	41	0.59
(2)	50	22	32	0.63
(3)	38	20	29	0.60
(4)	49	38	42	0.63
(5)	44	25	34	0.58
(6)	40	21	29	0.60
Mean	46.7	25.2	34.5	0.60
±1 *SD*	7.0	6.0	5.3	0.02

0022-3549/83/0600-0692$01.00/0

Table II—Hypoglycemic Effects of 1 U of Subcutaneously Administered Regular Insulin and Sodium Insulin

Assay No.	Nadirs of Blood Glucose Levels, mg/dl		Time of Nadir, min	
	Regular Insulin	Sodium Insulin	Regular Insulin	Sodium Insulin
(1)	32	47	100	148
(2)	46	40	90	190
(3)	27	43	130	195
(4)	24	42	130	195
(5)	50	48	100	145
(6)	45	32	80	105
(7)	38	41	80	162
Mean	37.4	41.8	101.4	162.9
±1 *SD*	10.1	5.3	21.2	33.4
p	*NS*		<0.001	

subcutaneously. The site of injection was always 2.54 cm from the center of the third nipple from the head. The glucose monitor measured the lowering of the blood glucose level. The nadir blood glucose level and the time of its occurrence were noted.

This procedure was repeated in each dog, after a rest period of 1 or 2 weeks, using 1 U of regular insulin instead of 1 U of sodium insulin.

RESULTS AND DISCUSSION

The results of the six repeated bioassays of a solution containing 0.6 U of sodium insulin as measured by immunoassay are shown in Table I. The mean biological activity of sodium insulin was equal to its immunoactivity. The coefficient of variation of the six consecutive immunoassays of sodium insulin was 3%.

The hypoglycemic effects of subcutaneously administered sodium insulin and regular insulin are compared in Table II. There was no significant difference between the nadirs of blood glucose following the two forms of insulin, but the nadir induced by sodium insulin occurred significantly later than the nadir induced by regular insulin.

Chemical manipulation of a biologically active molecule can have a significant effect on its biological as well as its immunological activity. The two effects may differ, leading to a new relationship between the two activities.

We have found that sodium insulin, a new dosage form of insulin, has the same ratio of biological *versus* immunological activity as the USP reference standard insulin.

Even though the biological effect of intravenously injected sodium insulin was equal to the effect of regular insulin, there was a significant difference between the effects of the two dosage forms of insulin when administered subcutaneously. The difference between the activity of subcutaneously administered sodium insulin and regular insulin, therefore, should be attributed only to the difference in the rate at which they are absorbed into the bloodstream.

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ACKNOWLEDGMENTS

Supported by Grant HD-16077 of the National Institutes of Health, United States Public Health Service. Sodium insulin and sterile insulin diluent, USP, were a gift from Dr. W. D. Storvick, Manager of Insulin Operation, Eli Lilly and Company. The regular insulin used was Illetin 100 U/ml from Eli Lilly and Company. The sodium insulin was batch 674-91GP-1-2.

Determination of Hydralazine in Human Whole Blood

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Received January 18, 1982, from the **College of Pharmacy, University of Texas-Austin* and the ‡*Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX 78284.* Accepted for publication June 2, 1982.

Abstract □ The time required for the separation of plasma from the cellular components of blood can permit the *in vitro* loss of hydralazine. Thus, a high-performance liquid chromatographic (HPLC) procedure for the measurement of hydralazine in blood has been developed. 4-Methylhydralazine was used as an internal standard. The addition of *p*-anisaldehyde led to the formation of the *p*-anisaldehyde hydrazones of hydralazine and the internal standard. HPLC on a reverse-phase cyano column provided an analytical procedure in which the average relative standard deviation over the concentration range of 1–160 ng/ml was 8.3%. Hydralazine pyruvic acid hydrazone, a known circulating metabolite of hydralazine, yielded only 0.05 mole % hydralazine when submitted to this assay procedure.

Keyphrases □ Hydralazine—whole blood, derivatization, *p*-anisaldehyde hydrazones of hydralazine, analysis, high-performance liquid chromatography □ Whole blood—analysis, hydralazine, high-performance liquid chromatography □ High-performance liquid chromatography—hydralazine, whole blood analysis, *p*-anisaldehyde hydrazone of hydralazine

Selective assays for the measurement of hydralazine in plasma have been recently described (1–3). A major disadvantage of these procedures is the time required for separation of plasma from the cellular components of blood. Hydralazine disappears rapidly from plasma or blood *in vitro* (2, 4–6). Rapid sample processing at reduced temperature has been used to slow the loss prior to derivatization (7–9). The measurement of hydralazine in whole blood would circumvent this problem. In addition, because the clearance of hydralazine is high (7, 8), blood hydralazine concentrations are extremely useful for pharmacokinetic studies. However, attempts to measure hydralazine in whole blood using previously published techniques for plasma (1, 3) resulted in fouling of the chromatographic column after only a few sample injections. This paper describes a high-performance liquid chromatographic (HPLC) assay for the determination of hydralazine in whole blood using a different chromatographic column and a less polar extraction solvent.

EXPERIMENTAL

Reagents and Chemicals—Hexane, methanol, and acetonitrile were purchased as glass-distilled solvents. The acetonitrile was filtered before

0022-3549/83/0600-0693$01.00/0