

Biological Activity of Alligator, Avian, and Mammalian Insulin in Juvenile Alligators: Plasma Glucose and Amino Acids

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The biological activity of alligator, turkey, and bovine insulin on plasma glucose and plasma amino acids was tested in fasted juvenile alligators. Preliminary experiments showed that the stress associated with taking the initial blood sample resulted in a hyperglycemic response lasting more than 24 hr. Despite repeated bleedings no additional hyperglycemic events occurred, and blood glucose declined slowly over the next 7 days. Under these conditions the smallest dose of insulin eliciting a hypoglycemic response was 40 $\mu\text{g}/\text{kg}$ body wt. A dose of 400 $\mu\text{g}/\text{kg}$ body wt of either alligator or bovine insulin caused a pronounced hypoglycemia by 12 hr postinjection. Maximum decline in plasma glucose occurred at 24 to 36 hr with a slow return to control levels by 120 hr. There were no significant differences in the hypoglycemic responses to any of the three insulins tested. The decline in plasma amino acids was much more rapid than the decline in plasma glucose in response to insulin. Even at the 40 $\mu\text{g}/\text{kg}$ body wt dose a significant difference from saline-injected control was seen at 2 hr postinjection. Maximum decline in plasma amino acids occurred at 8 to 12 hr with a return to baseline by 36 hr. These results show that the relatively conservative changes in the sequence of alligator insulin (three amino acid substitutions in the B-chain compared with that of chicken) have little effect on biological activity and that alligator insulin receptors do not appear to discriminate among the three insulins. © 1993 Academic Press, Inc.

There have been a number of studies on the structure of the endocrine pancreas of crocodylians, some of which have included detailed descriptions of the putative hormone secreting cells (Titlbach, 1969a,b, 1981; Gabe, 1970; Miller and Lagios, 1970). Endocrine cells of the crocodylian pancreas do not occur in discrete islets, as is the case in mammals, but in cords, clumps of cells, or even individual cells interspersed among the exocrine tissue (Titlbach, 1969a, 1981; Buchan *et al.*, 1982). Ultrastructural studies of the endocrine pancreas of alligator (Titlbach, 1981; Buchan *et al.*, 1982), black caiman (Titlbach, 1981), dwarf crocodile (Grillo *et al.*, 1976), and Nile crocodile (Rhoten, 1987) provided immunocytochemical evidence of the four known pancreatic hormones in crocodylians, three of which

were isolated and sequenced from alligator pancreas (Lance *et al.*, 1984).

There have, however, been very few physiological studies on pancreatic endocrine function in crocodylians. The first reported experiment on the effect of mammalian insulin on blood glucose in a crocodylian was that of Houssay and Rietti (1924). The exact numbers were not given, but they reported a marked hypoglycemia after injecting young *Caiman sclerops* (120 to 720 g) with 10 to 60 units of insulin. Hypoglycemia was not evident until 24 hr after treatment and reached a nadir in 3 to 4 days. At this time they noted hyperexcitability, trembling, and convulsions. Although blood sugar showed a slight increase after 8 days, all the animals died by 10 days (Houssay and Rietti, 1924). Total

pancreatectomy resulted in severe hyperglycemia and a permanent diabetic state in the alligator, and mammalian insulin was able to restore blood sugar to close to normal levels in these pancreatectomized alligators (Penhos *et al.*, 1967). Ovine insulin (0.25 U/kg) given to intact alligators produced a maximum decline in blood glucose from 82 to 16 mg% by 24 hr and a slow (12 day) recovery to 87 mg% (Penhos *et al.*, 1967). Coulson and Hernandez (1953) reported that the smallest dose of insulin giving a measurable effect in a glucose-loaded alligator was 0.5–1.0 U/kg, but noted that the dose of insulin required to induce hypoglycemia in a fasted animal was not necessarily the same as that required to produce a significant effect after the injection of glucose (Coulson and Hernandez, 1964). Stevenson *et al.* (1957) stated that 10 units of insulin/kg body wt. was the normal effective dose in an alligator. Pharmacological doses of insulin (up to 1000 U/kg) in the alligator resulted in a rapid hyperglycemic response followed by a prolonged hypoglycemia and coma (Coulson and Hernandez, 1964).

The effect of mammalian insulin on amino acid metabolism in the alligator and caiman has been shown to be essentially similar to that in mammals, a decline in plasma amino acids, albeit at a much slower rate, and presumably an increase in protein synthesis. However, the doses of insulin needed to produce this effect are an order of magnitude greater than those required in mammals (Hernandez and Coulson, 1961, 1968, 1969; see also Coulson and Hernandez, 1983, for review).

It is not known if the relative insensitivity of alligators to mammalian insulin with respect to plasma amino acids and plasma glucose is due to species specificity because of structural differences in their respective insulins, or simply due to the relative insensitivity of reptiles to insulin in general (Miller, 1960; Gabe, 1970; Penhos and Ramey, 1973; Sidorkiewicz and Skoc-

zylas, 1974). Alligator insulin has been isolated and the amino acid sequence determined (Lance *et al.*, 1984), but the biological activity of alligator insulin has not been tested. The A-chain of alligator insulin is identical to that of chicken insulin and the B-chain differs from that of chicken in only three positions. The three substitutions are relatively conservative and would not be expected to have a significant effect on the tertiary structure (Blundell *et al.*, 1972) and hence, on the biological activity. To test this hypothesis the biological activities of alligator, chicken, turkey (identical to chicken), ovine, and bovine insulins on blood glucose and amino acids were tested in fasted juvenile alligators. Part of this work was reported previously (Lance *et al.*, 1984).

MATERIALS AND METHODS

An initial experiment was conducted at the Rockefeller Wildlife Refuge, Grand Chenier, Louisiana in an attempt to establish a dose response to various insulins. Subsequent experiments were carried out in the Department of Biochemistry, LSU School of Medicine, New Orleans and at the Rockefeller Wildlife Refuge.

Seventy-eight juvenile alligators, mean body weight 208 g (range 85–680 g) were captured at night by hand from an airboat at the Rockefeller Wildlife Refuge and held in rearing chambers maintained at 28° for 1 week prior to the experiment (see Joanen and McNease, 1979, for details). Food was withdrawn 72 hr before the initial blood sample was taken, and the following insulin preparations were tested for hypoglycemic activity: porcine insulin (Sigma), chicken insulin (generously donated by the late Joe Kimmel, University of Kansas School of Medicine), and alligator insulin (Lance *et al.*, 1984). All insulins were dissolved in physiological saline and injected intraperitoneally at 0.01, 0.1, 1.0, and 10 µg per animal, uncorrected for body weight. Six alligators/group were injected. Assuming 24 U/mg bovine insulin, and assuming that the dose (0.25 U/kg) administered by Penhos *et al.* (1967) was supraphysiological, the maximum dose of 10 µg in a 200-g alligator should be approximately 1.20 U/kg, four to five times the dose administered by Penhos *et al.* (1967). Immediately before injection an initial blood sample was collected via heart puncture using a 1-cc heparinized syringe fitted with a 26-g needle. Subsequent blood samples were taken at 1, 2, 4, 8, 12, 24, 48, 72, 144, 216, and 288 hr postinjection. Alligators were

maintained without food at 28° throughout the sampling period. Red cells were separated by centrifugation, and the plasma was stored at -20° until assayed colorimetrically for glucose by the glucose oxidase method (Sigma).

In addition to the above, a second group of 42 wild-caught alligators (mean body weight 259 g, range 85–709 g) were bled in groups of six at 4-hr intervals for 28 hr to test for any circadian variation in blood glucose. Each alligator was bled only once in this experiment.

When it was noted that the doses tested in the initial experiment failed to cause hypoglycemia (see Results), a second experiment, in which nine alligators raised at the LSU School of Medicine since hatching (mean body weight 465 g, range 334–545 g) were injected with 400 µg/kg body weight of alligator ($n = 3$) or bovine insulin ($n = 3$), or saline ($n = 3$), was carried out. The animals were bled via the tail vein at 0, 1, 2, 4, 6, 8, 12, 16, 24, 30, 36, 48, and 72 hr, and the plasma was assayed for glucose.

An additional set of experiments using slightly older and heavier alligators (mean body weight 1006 g, range 610–1760 g, $n = 50$) was carried out at the Refuge. These animals were hatched from artificially incubated eggs at the Rockefeller Wildlife Refuge, maintained in constant temperature incubators at 28°, and fed a diet of ground nutria (*Myocastor coypus*) supplemented with a poultry vitamin mix (Joanen and McNease, 1979). Food was withdrawn 10 days prior to the start of the experiments. Zinc-free alligator insulin, turkey insulin (TCT Prep NB 1, from J. Kimmel), and bovine insulin (Eli Lilly, Lot 615-70N-80) were dissolved in 0.9% saline and injected intraperitoneally into the alligators immediately after the initial blood sample was drawn. Injection volumes were adjusted for body weight such that each animal received 40, 100, or 200 µg/kg body wt of the respective insulins. Control alligators received saline alone, $n = 5$ for each group. Blood samples were collected via heart puncture at 1, 2, 4, 6, 8, 12, 16, 24, 36, 48, 60, 72, 96, and 120 hr, and an aliquot of plasma was frozen for glucose assay. For the amino acid assay, 0.1 ml of freshly collected plasma was added to 2 ml of 95% ethanol. The ethanolic mixture was centrifuged and the supernatant was decanted into clean glass vials until it was assayed for total amino acids using the Technicon amino acid analyzer as described in Coulson and Hernandez (1968).

The effect of temperature on the response of alligators to insulin was carried out using animals maintained at the LSU Medical School since hatching, with mean body weight 823g (range 800–850g). Alligators were held for 24 hr at 25° ($n = 4$) or 28° ($n = 4$) in a controlled environmental chamber before the start of the experiment and maintained at either 25° or 28° for the duration of the experiment. Each animal was injected with 400 µg/kg body wt (10 U/kg) of bovine insulin immediately after the initial blood sample was taken. Subsequent blood samples were collected from

the tail vein at various intervals up to 120 hr after injection of insulin. The blood was centrifuged and the plasma was separated and placed in ethanol as above until it was assayed for glucose and total amino acids.

The data from the dose response experiments were subjected to a repeated measures single factor ANOVA followed by the Scheffe's multiple range test, and by a factorial ANOVA followed by Scheffe's multiple range test for the individual time periods. Significance level was 95%. The circadian data subjected to a single factor ANOVA. The calculations were carried out using the Statview software package on a Macintosh personal computer.

RESULTS

Blood glucose in the 78 wild-caught alligators fasted for only 72 hr ranged from 43 to 264 mg% (mean = 134 ± 5 SEM). The disturbance caused by attempting to bleed all the animals in a single environmental chamber within a limited time period resulted in a stress response and thus higher blood glucose in the alligators sampled several minutes after those bled immediately. In all groups of alligators plasma glucose was seen to rise significantly between 8 and 12 hr after injection ($P < 0.001$) in response to the stress of handling and blood sampling (Fig. 1 and Tables 1, 2, and 3). None of the insulins tested in the initial experiments caused any significant hypoglycemia at any dose, but porcine and alligator insulins at the 10-µg dose did blunt the stress-induced hyperglycemia. Significant differences from control were noted only at the 24-hr period ($P < 0.05$, Tables 1 and 3) for alliga-

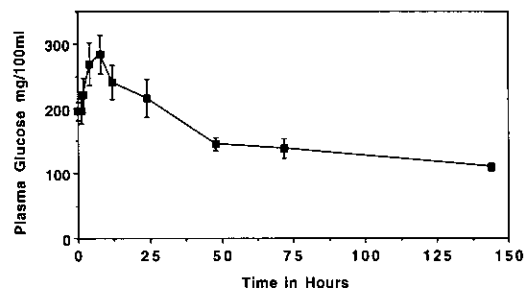


FIG. 1. Stress-induced rise in plasma glucose in saline-injected alligators ($n = 6$). Plasma glucose continued to decline reaching 80 ± 7 mg/100 ml by 288 hr (data not shown). Solid square represents the mean, and the bars the standard error of the mean (SEM).

TABLE 1
EFFECT OF ALLIGATOR INSULIN ON BLOOD GLUCOSE IN THE ALLIGATOR

Time (hr)	Saline	Dose injected			
		0.01 μg	0.10 μg	1.00 μg	10.0 μg
Initial	196 \pm 14 ^a	112 \pm 10	108 \pm 5	134 \pm 20	105 \pm 10
1	196 \pm 20	110 \pm 9	94 \pm 10	116 \pm 18	100 \pm 12
2	221 \pm 26	120 \pm 12	105 \pm 9	148 \pm 12	81 \pm 15
4	269 \pm 33	192 \pm 20	138 \pm 14	285 \pm 38	138 \pm 24
8	284 \pm 29	241 \pm 24	197 \pm 11	305 \pm 25	149 \pm 20
12	241 \pm 26	219 \pm 20	162 \pm 20	290 \pm 17	126 \pm 21
24	217 \pm 30	170 \pm 17	136 \pm 4	225 \pm 19	103 \pm 27 ^b
48	145 \pm 10	119 \pm 8	107 \pm 9	147 \pm 14	128 \pm 13
72	155 \pm 8	123 \pm 5	125 \pm 10	122 \pm 20	112 \pm 9
144	110 \pm 6	141 \pm 8	141 \pm 6	143 \pm 15	117 \pm 7
216	98 \pm 4	128 \pm 6	109 \pm 11	152 \pm 5	111 \pm 10
288	80 \pm 7	85 \pm 5	82 \pm 4	109 \pm 7	88 \pm 8

^a Values represent the mean \pm SEM.

^b Significantly different from all other groups ($P < 0.05$).

tor insulin and at 8, 12, and 24 hr for the porcine insulin. The response to chicken insulin (Table 2) was indistinguishable from control at all doses and at all times. By 216 hr the plasma glucose in the saline-injected animals had returned to normal levels, and there were no differences between any of the groups (Tables 1, 2, and 3).

The results of the circadian study (Fig. 2) indicated that the stress-induced hyperglycemia masked any possible underlying circadian variation in blood glucose.

Injection of 400 $\mu\text{g}/\text{kg}$ of either bovine or alligator insulin produced no significant differences in the hypoglycemic response to the two insulins at any of the times postinjection (Fig. 3). Both insulins caused a significant decline in plasma glucose by 8 hr ($P < 0.01$) and both resulted in significantly lower glucose than the initial level throughout the experiment. Glucose levels in both groups (92 mg%) were close to fasted levels by 72 hr.

In the experiment using older and heavier

TABLE 2
EFFECT OF CHICKEN INSULIN ON BLOOD GLUCOSE IN THE ALLIGATOR

Time (hr)	Saline	Dose injected			
		0.01 μg	0.10 μg	1.00 μg	10.0 μg
Initial	196 \pm 14	133 \pm 7	130 \pm 6	167 \pm 36	171 \pm 17
1	196 \pm 20	151 \pm 15	138 \pm 9	198 \pm 21	174 \pm 16
2	221 \pm 26	147 \pm 28	148 \pm 14	217 \pm 35	174 \pm 17
4	269 \pm 33	208 \pm 24	195 \pm 16	284 \pm 38	243 \pm 17
8	284 \pm 29	209 \pm 24	172 \pm 10	237 \pm 33	231 \pm 34
12	241 \pm 26	228 \pm 34	165 \pm 9	260 \pm 23	225 \pm 40
24	217 \pm 30	152 \pm 25	134 \pm 6	212 \pm 35	192 \pm 34
48	145 \pm 10	137 \pm 3	122 \pm 3	142 \pm 15	126 \pm 14
72	155 \pm 8	124 \pm 5	121 \pm 5	141 \pm 10	106 \pm 7
144	110 \pm 6	140 \pm 4	136 \pm 2	133 \pm 6	137 \pm 6
216	98 \pm 4	105 \pm 6	104 \pm 7	108 \pm 18	114 \pm 17
288	80 \pm 7	78 \pm 9	84 \pm 3	97 \pm 8	101 \pm 6

TABLE 3
EFFECT OF PORCINE INSULIN ON BLOOD GLUCOSE IN THE ALLIGATOR

Time (hr)	Dose injected				
	Saline	0.01 µg	0.10 µg	1.00 µg	10.0 µg
Initial	196 ± 14	120 ± 19	125 ± 14	144 ± 12	91 ± 11
1	196 ± 20	118 ± 20	101 ± 13	156 ± 13	110 ± 18
2	221 ± 26	127 ± 28	105 ± 15	149 ± 15	85 ± 16
4	269 ± 33	207 ± 34	212 ± 18	262 ± 19	161 ± 25
8	284 ± 29	220 ± 27	233 ± 15	250 ± 27	144 ± 26
12	241 ± 26	170 ± 17	209 ± 19	256 ± 37	124 ± 20
24	216 ± 30	104 ± 13	212 ± 10	154 ± 23	80 ± 16
48	145 ± 10	128 ± 16	127 ± 9	178 ± 11	102 ± 26
72	139 ± 15	127 ± 5	121 ± 9	123 ± 1	87 ± 32
144	110 ± 6	123 ± 8	139 ± 7	146 ± 5	142 ± 7
216	98 ± 4	104 ± 13	106 ± 12	126 ± 19	132 ± 5
288	80 ± 7	92 ± 14	97 ± 7	109 ± 21	98 ± 12

alligators fasted for 10 days, blood glucose at the initial sampling was still relatively high, ranging from 83 to 218 mg% (mean = 139 ± 5 SEM, n = 50), suggesting again that a stress-induced increase in blood glucose had occurred. Each of the three insulins tested was able to overcome this hyperglycemia and cause a significant decrease in blood glucose (Figs. 4a, 4b, and 4c). Bovine insulin caused significant hypoglycemia by 12 hr even at the 40 µg/kg dose (P < 0.05), whereas turkey and alligator insulin were ineffective at the 40 µg/kg dose. Doses of bovine insulin at 100 and 200 µg/kg caused hypoglycemia persisting for the duration of the experiment with glucose levels rising to only 78 mg% at 120 hr. Turkey insulin caused significant hypoglycemia (P < 0.05)

but only at 16 and 24 hr with 100 and 200 µg/kg. Alligator insulin caused significant hypoglycemia only at 24 hr (P < 0.05) with the 100 µg/kg dose and at 24 and 36 hr with the 200-µg/kg dose.

Plasma amino acids declined significantly in response to only 40 µg/kg of both turkey and alligator insulin by 2 hr postinjection (P < 0.05). The response to 40 µg/kg bovine insulin was not significant until 6 hr postinjection, and maximum response to 40 µg/kg of all three insulins occurred between 8 and 16 hr. Maximum response to 100 µg/kg alligator insulin occurred at 36 hr postinjection, but between 12 and 24 hr for the 100-µg/kg dose of bovine and turkey insulins (Figs. 5a, 5b, and 5c).

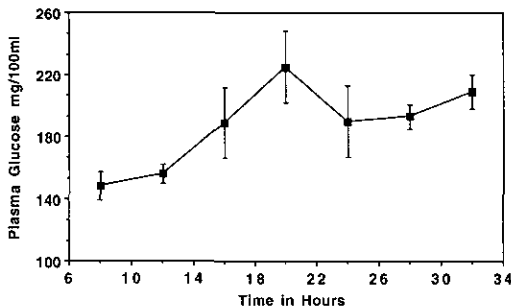


FIG. 2. Plasma glucose in different groups (6 per group) of juvenile alligators bled at 4-hr intervals. Means and SEM as in Fig. 1.

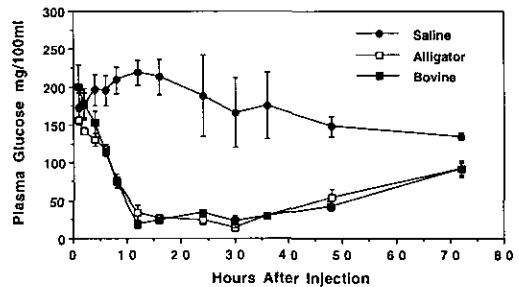


FIG. 3. Plasma glucose in alligators after a single injection of saline (n = 4), 400 µg/kg wt of bovine (n = 4) or alligator (n = 4) insulin. Means and SEM as in Fig. 1.

The effect of temperature on the response to 400 μg (10 units) of bovine insulin in alligators is shown in Fig. 6. At 28° the fall in plasma glucose was significantly faster than at 25°. By 10 hr plasma glucose in the 28° group was significantly lower ($P < 0.05$) than the initial sample, whereas glucose in the 25° group was not significantly different from the initial level until 24 hr ($P < 0.05$). Similarly, the time to return to baseline was also slightly faster at the higher temperature. The fall in plasma amino acids in response to insulin was not significantly faster at the higher temperature, both groups were significantly different from the initial at 6 hr ($P < 0.05$).

DISCUSSION

Hyperglycemia in response to the stress

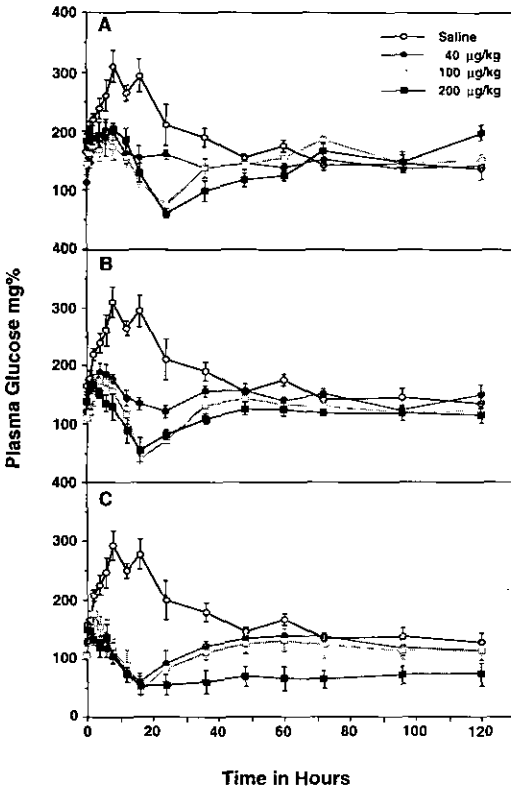


FIG. 4. Plasma glucose in alligators after a single injection of alligator insulin (A), turkey insulin (B), or bovine insulin (C) at three different concentrations. Five animals per group. Each point represents the mean, and the bars represent the SEM.

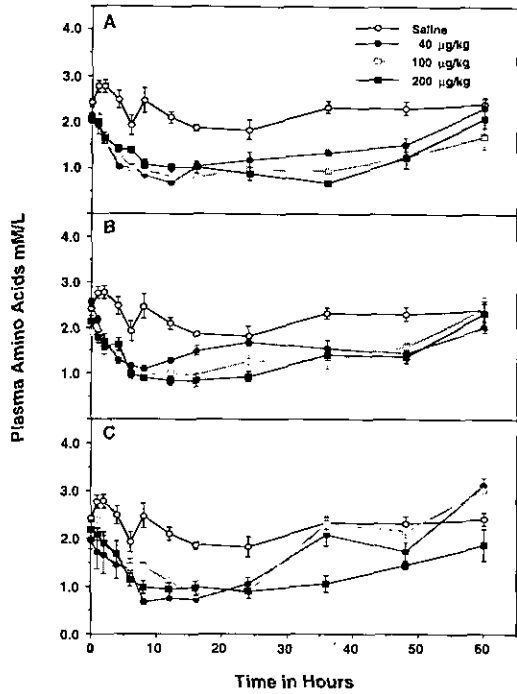


FIG. 5. Plasma amino acids in alligators after a single injection of alligator (A), turkey (B), or bovine (C) insulin. Same animals as in Fig. 4, but values beyond 60 hr were not included as there were no significant differences between the saline- or insulin-injected values beyond this time.

of handling and bleeding in alligators is similar to what has been reported in other reptiles (Prado, 1946; Skoczylas and Sidoriewicz, 1974) and is a well-known response in fish (Morales *et al.*, 1990). The mechanism for this rise in alligators is not certain. It is unlikely to be due to increased secretion of corticosterone seen in response to stress (Gist and Kaplan, 1976; Lance and Lauren, 1984; Lance and Elsey, 1986), as repeated injections of pharmacological doses of hydrocortisone cause only mild hyperglycemia in alligators (Coulson and Hernandez, 1953). The stress-induced hyperglycemia in alligators may be due to catecholamine secretion. In fish there is a correlation between loss of liver glycogen and hyperglycemia in response to stress, effects that can be mimicked by injections of catecholamines (Pickering, 1981), and a

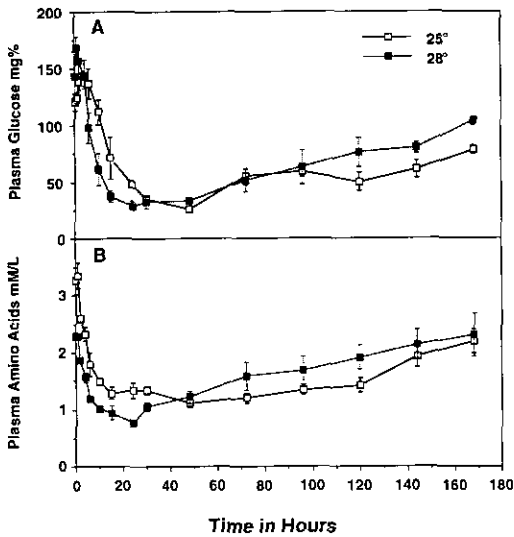


FIG. 6. Plasma glucose (A) and plasma amino acids (B) in alligators injected with 400 $\mu\text{g}/\text{kg}$ bovine insulin held at 25° ($n = 4$) and at 28° ($n = 4$).

positive correlation between plasma catecholamines and glucose (Nakano and Tomlinson, 1967). There are no data on circulating levels of catecholamines in alligators, but injections of epinephrine do cause significant hyperglycemia by 8 hr postinjection (Stevenson *et al.*, 1957), a similar time to peak hyperglycemia seen during handling stress. It is interesting to note that despite the repeated stress of handling and blood sampling there was only a hyperglycemia response after the initial sampling period. Evidently repeated stressful encounters do not result in repeated surges of catecholamines, or the initial challenge depletes the animal's glycogen stores.

In the first part of this study 0.1 to 10 μg of insulin (ca. 0.12 to 1.2 U/kg body wt) injected into wild-caught alligators did not produce a marked hypoglycemic effect, but did show that relatively small doses of insulin can cause a significant diminution of the stress-induced hyperglycemia. Plasma glucose in the groups treated with 10 μg of porcine and alligator insulin was significantly lower at 24 hr postinjection than in groups injected with chicken insulin or sa-

line. The apparent lack of biological activity of chicken insulin is not clear but may have been due to deterioration of the preparation.

The reason for the apparent insensitivity of reptiles to insulin may be due to the stress-induced hyperglycemia. The early reports claiming an initial hyperglycemic effect of insulin in reptiles (Coulson and Hernandez, 1953; Gabe, 1970) are probably an artifact due to glucagon contamination of the insulin. Penhos and Ramey (1973), using a highly purified preparation of insulin, reported no initial hyperglycemic response in the alligator. We conclude from our results and those of Coulson and Hernandez (1983) that alligators may be insensitive to doses considered physiological in a mammal, but are sensitive to doses as low as 1 U/kg. The dose of 0.25 U/kg reported by Penhos *et al.* (1967) to cause a prolonged hypoglycemia in the alligator is difficult to reconcile with our results. Penhos and Ramey (1973) discussed the relative insensitivity of reptiles to insulin and reported the effects of injections of 1 and 10 U/kg of mammalian insulin in fasted alligators. In their hands an injection of 1 U/kg caused a prolonged (6–7 day) hypoglycemia. They did not present data from saline-injected controls. Our experiment, in which 200 $\mu\text{g}/\text{kg}$ (10 units) of bovine and alligator insulins was injected into alligators, gave a response similar to that reported by Penhos and Ramey (1973) with 1 U/kg. One possibility is that the alligators used by Penhos and Ramey had depleted their glycogen stores, did not exhibit the stress-induced hyperglycemia, and thus were more sensitive to insulin.

In our second set of experiments, 40 $\mu\text{g}/\text{kg}$ of bovine insulin did cause a significant hypoglycemia and appeared more potent than alligator insulin. This apparent lesser potency of alligator insulin remains equivocal until the purities of the various preparations are compared. The presence of zinc in the bovine preparation is unlikely to have

resulted in increased potency as it has been shown that insulin is no longer associated with the zinc 60 sec after secretion in rats (Gold and Grodsky, 1984).

A decrease in plasma amino acids in response to bovine, turkey, and alligator insulins was evident by 2 hr postinjection, whereas plasma glucose did not show a significant decline until 8 and 12 hr postinjection. The reason for this apparent difference in the glucose and amino acid response is probably the result of the difference in the response to stress; plasma amino acids were unaffected whereas glucose was significantly increased. The insulin effect on glucose was slower than that on amino acids due to the stress-induced hyperglycemia masking its early biological action. In contrast to the hypoglycemic response, alligator and turkey insulins were if anything more active than the bovine insulin on amino acid metabolism.

Our results suggest that alligator insulin is not any more active than mammalian or avian insulin on glucose and amino acid metabolism in the alligator. Black *et al.* (1963) suggested that the metabolic activity of the alligator depends to a greater extent on the utilization of fatty acids than of carbohydrates. Plasma free-fatty acids are extremely low (<5 mmol) in fasted alligators (Lance, unpublished), but the effect of insulin on plasma lipids has not been tested. Thus alligator insulin may be more effective than mammalian insulin in controlling lipid metabolism in the alligator. However, the structural similarity of alligator and mammalian insulins argues against this possibility, since the amino acid substitutions in alligator insulin are relatively conservative and would not be expected to cause any major conformational change in the molecule (Blundell *et al.*, 1972). The fact that insulin receptors in alligator liver and brain bind mammalian insulin with similar affinities to those seen in rat tissues (Shemer *et al.*, 1987) reinforces this view. Shemer *et al.* (1987) concluded that insulin receptors

in alligator liver and brain were more like those of the rat than those of the lizard in their binding characteristics, subunit nature, and approximate molecular weight.

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