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Insulin Effects on Positively-Reinforced Lever Pressing by Rats[1]

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GROVE, R. N. AND T. THOMPSON. *Insulin effects on positively-reinforced lever pressing by rats.* PHARMAC. BIOCHEM. BEHAV. 2(1) 35–44, 1974. – Regular insulin in doses greater than 0.1 units/kg produced dose-dependent maximum decrease in plasma glucose levels of food deprived rats 60 minutes after injection. FR 1 food reinforced lever pressing showed a dose-dependent, food deprivation-dependent and pretreatment time-dependent decrement in operant responding. Insulin suppression of responding was reduced when subjects were partially food satiated. FR 1 water reinforced behavior also showed a dose-dependent and pretreatment time-dependent decrement in lever pressing. VI 1 min food reinforced level pressing similarly showed a dose-dependent and pretreatment time-dependent decrement in responding. Thus, effects of insulin on operant responding were largely independent of type of reinforcer and schedule of reinforcement. Further, the failure to observe a consistent increase in responding with insulin did not support the view that insulin induces a state comparable to increased food deprivation levels. These findings suggest that the spectrums of effects of insulin and food deprivation differ.

Insulin Hunger Blood sugar Positively reinforced behavior Operant behavior

INSULIN is intimately involved in carbohydrate metabolism and regulation of blood sugar levels. As a consequence, insulin has been thought to play a major role in the regulation of food intake [7]. Because it increases food consumption, it has been suggested that insulin may also increase the rate of operant responding maintained by food reinforcement [7]. Morgan and Morgan [10,11] did not find that insulin induced operant lever press rate increases in normal food deprived rats; the opposite effect was observed, a rate decrement. Booth and Brookover [2] reported an increased rate of lever pressing reinforced on a fixed interval 15 sec schedule but a decrease in a fixed ratio 10 schedule of food reinforced responding following insulin administration. Insulin increased response rate of rats in the punished component of a multiple FR 30-food, FR 3-sucrose plus shock schedule, where either a standard Noyes food pellet or a sucrose pellet paired with a brief shock were the consequence during each component [16]. Balagura and Hoeble [1] found that responding for electrical lateral hypothalamic stimulation increased by about 20% above preinjection control rates after insulin. The same sites elicited feeding when stimulated.

The foregoing findings indicate that few generalizations can be made concerning the effects of insulin on operant behavior. Both rate increases and decreases have been reported, though in studies involving free access to food, insulin generally increases food intake over a wide dose range (10–75 units/kg) [2]. The present investigation explored the effects of insulin dose, pretreatment time, the type of reinforcer (i.e. food or water), schedule of reinforcement and level of food deprivation on operant lever pressing. The purpose was to clarify the discrepancies in the literature concerning the effects of insulin on lever pressing maintained by food and water reinforcement.

EXPERIMENT I: PLASMA GLUCOSE DOSE-RESPONSE RELATIONS FOR INSULIN IN FOOD DEPRIVED RATS

This study was conducted to establish an insulin–blood glucose dose-response curve in food deprived rats. Similar food–deprivation and insulin treatments were used in subsequent studies in which change in operant lever pressing was the dependent variable.

METHOD

Animals

Four Holtzman male albino rats approximately 6

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months old at the beginning of this experiment were used. At 85 percent of their free feeding weights Rat B2 weighed 435 g; Rat B4, 440 g; Rat D5,465 g and Rat D6, 540 g.

Procedure

These animals were maintained at 85 percent of their free feeding body weights. Either saline or regular insulin solutions were administered subcutaneously on alternate days. U-40 Regular Insulin (Lilly) was suspended in a 0.5 ml saline vehicle immediately before injection. Three doses of insulin were used: 0.1, 1.0, and 10.0 insulin units per kilogram (u/kg). Each dose was administered twice to each animal in one of four orders, one order per animal.

Blood samples were obtained immediately before the session and 30, 60 and 240 min following injection by the tail-clip method [18]. Sample acquisition took 3 to 5 min after which animals were returned to a carrying cage. Plasma glucose levels were obtained using the Calbiochemical Glucose Hexokinase Assay Procedure [13]. Following each session, animals were returned to home cages and fed and watered. At the highest insulin dose, 10 u/kg, convulsions were occasionally observed. On these occasions, the session was immediately terminated with the i.p. injection of 1 ml of 50% dextrose solution.

RESULTS

The mean blood glucose levels for each animal were calculated at each dose across the four pretreatment intervals, unless a subject convulsed thus terminating that session. Convulsions always occurred before the final interval at the 10 u/kg dose for three of the four subjects.

Dose-time curves for each animal are shown in Fig. 1. Preinjection plasma glucose levels varied between animals from 94 to 140 mg/100 ml. In general, there was a slight decline in plasma glucose levels across the saline sessions. The lowest dose of insulin, 0.1 u/kg, produced an effect similar to that of saline. At 1.0 u/kg plasma glucose levels declined to a minimum of 35 to 52 mg/100 ml. This ef-

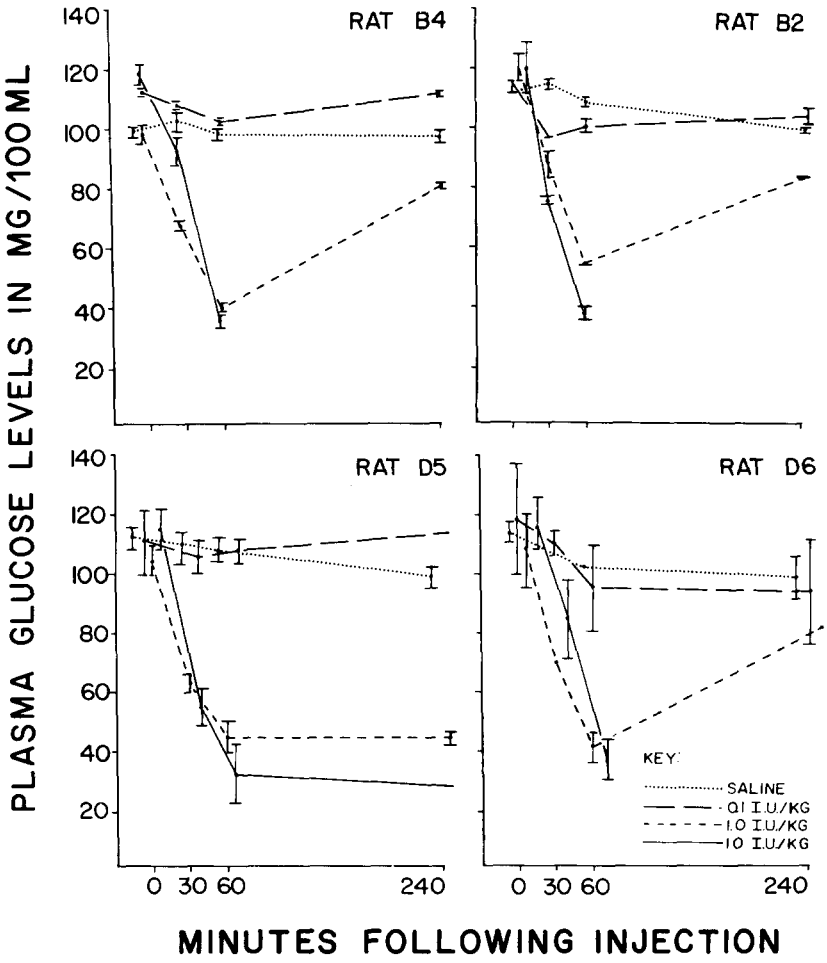

FIG. 1. Dose time curves for plasma glucose levels for four rats (B4, B2, D5 and D6). Ordinate: plasma glucose levels (mg/100 ml). Abscissa: minutes following injection. Points represent mean blood gluocse levels, and vertical bars indicate ranges. Data points without vertical bars indicate no replication was made.

fect was greatest 60 min following injection; three animals, rats B2, B4 and D6, then showed a gradual return of plasma glucose levels toward saline values 240 min following 1.0 u/kg. At the 10.0 u/kg dose plasma glucose levels declined to 23 to 45 mg/100 ml 60 min following injection. However, only one animal (D5), survived the fourth hour for one administration of the 10.0 u/kg dose. For this animal blood levels remained suppressed at 27 mg/100 ml at the end of the four hr session. A (two-between) × (one-within) groups additive model analysis of variance [9] revealed a significant difference among blood glucose levels due to insulin dose ($F(3,9) = 61.38$, $p<0.001$), time of measurement ($F(3,9) = 68.98$, $p<0.001$) and dose × time of measurement interaction effects ($F(9,27) = 14.67$, $p<0.001$). In general, both the extent and duration of hypoglycemia varied directly with increasing doses.

DISCUSSION

Insulin in doses of 1.0–10.0 u/kg produced a marked decrease in plasma glucose while the lowest dose, 0.1 u/kg, produced an effect on blood glucose level not unlike that found with saline. These data allowed some estimation of appropriate doses for food deprived rats in the following behavioral experiments.

These results generally agree with those found in similar rat studies [1,14]. It should be noted that the effective range for insulin hyperphagia in rats is 10–75 u/kg, s.c. of regular insulin [2], doses which are considerably higher than the minimally effective hypoglycemic dose reported here: 1.0 u/kg. Steffens [14] also found that free-fed rats injected subcutaneously with 0.6 units per animal (about 2.4 u/kg) became hypoglycemic but not hyperphagic. Therefore, hyperphagia may be elicited at doses above that necessary to produce only hypoglycemia, assuming that food was freely available. For these reasons, a dose range from about 0.1 to 60.0 units/kg was examined in the following operant studies.

EXPERIMENT II: THE EFFECTS OF INSULIN ON FIXED RATIO 1 FOOD REINFORCED LEVER PRESSING

The present study replicated the Morgan and Morgan [10,11] studies with normal rats, exploring the dose-response and pretreatment time relations. This investigation also examined the sensitivity of this behavioral preparation to insulin under two levels of food deprivation.

METHOD

Animals

Three naive Holtzman male albino rats approximately 7 months old at the beginning of this experiment were used. During the initial high food deprivation treatment conditions where animals were 23 hours food deprived, Rat I3 weighed 480 g; Rat A5, 395 g and Rat A6, 375 g. These weights were approximately 80 (±5) percent of their free-feeding body weights.

Apparatus

Standard Gerbrands rat operant conditioning chambers enclosed in sound attenuating compartments were used. In each chamber two Gerbrands rat levers positioned 10 cm above the floor of the cage were mounted on one wall of a 23 × 20 × 18 cm Plexiglas and aluminum experimental chamber. One stimulus light was located above each lever. The feeder magazine was placed on the right side of the recess between the two levers at the level of the cage floor. During each session, a light above each was illuminated and bar presses on the right lever only were recorded and fed into the recording apparatus in an adjoining room. When reinforcement was scheduled, a bar press on the right lever operated a pellet dispenser delivering a 45 mg Noyes rat food pellet into the magazine. White noise was present inside the chamber and the chamber lights were programmed to extinguish for the 2 sec duration of the feeder operation.

Procedure

Following initial lever press training on a FR 1 reinforcement schedule, where each response was reinforced, animals were allowed access to food only during the daily 50 min experimental sessions. Water was freely available in the home cage but not in the experimental chamber. Experiments were conducted daily until not more than ±10% variability in daily response rates was observed over three succeeding days.

During the first phase (Phase A) animals were maintained solely on food pellets presented following lever presses. This was designated as the high deprivation condition since food was available only during the 50 min sessions each day. Saline and insulin treatments were administered subcutaneously on alternate days except when the baseline was disrupted in which case further saline trials were given. This rarely occurred. The order of dosage was unsystematic and dosage levels (0.1, 1.0, 10.0 and 40.0 u/kg) were replicated at least once and in some instances, twice. The initial dose-response relation was obtained using a 30 min pretreatment time. A second dose-response relation was obtained using a 30 min pretreatment time. A third dose-response relation was obtained using a 60 min pretreatment interval.

The second phase (Phase B) will be referred to as the low deprivation condition; approximateiy 20 g of food was given in the home cage following each session increasing body weights to approximately 95% of their free-feeding body weight. Following stabilization of responding in Phase B the insulin treatment schedule was then repeated, first at a 30 min, then a 60 min pretreatment interval.

RESULTS

Figure 2 shows the individual mean lever pressing rate and saline control ranges as a function of insulin dose at the 30 and 60 min pretreatment times under the two deprivation conditions, A and B.

Under high food deprivation conditions (A) the response rate changed very little following insulin at 30 min pretreatment time; however, at the 60 min pretreatment time insulin produced a more pronounced shift in the rate of responding; the major effect was a dose-dependent decrease in responding with nearly complete response suppression at 1.0 u/kg for Rats A5 and A6 and 10.0 u/kg for I3.

When allowed access to food sufficient to maintain them at 95% of their free-feeding body weight (B) saline

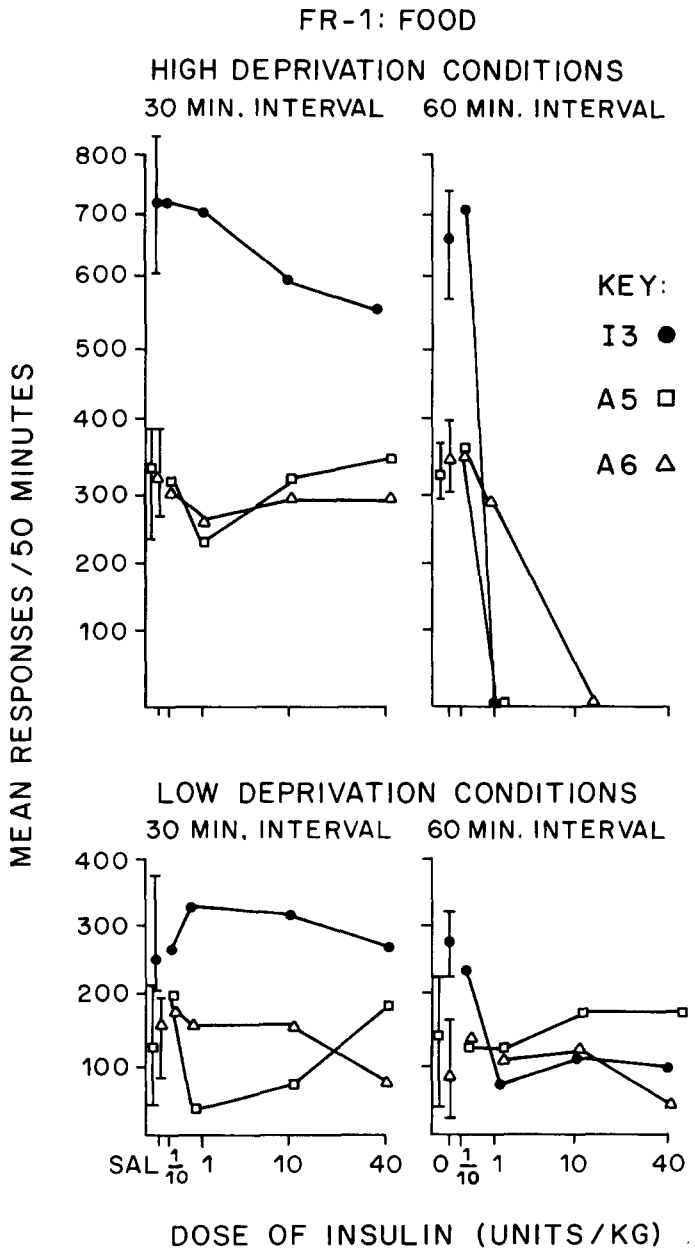

FIG. 2. Dose response curves for fixed ratio 1 food reinforced performance under high and low food deprivation conditions. Top graphs indicate high deprivation effects, lower graphs indicate low deprivation effects. Left graphs indicate effects for 30 min pretreatment time, right graphs for 60 min pretreatment time. Points are means, bars are control ranges.

control lever pressing rates were considerably lower and more variable. Following the 30 min insulin pretreatment the temporal pattern and overall lever press rate remained variable across doses, but ranged within control values. A similar effect occurred under low deprivation when injected 60 min prior to the session; there was less evidence of insulin induced suppression at either 10.0 or 40.0 u/kg. This is in marked contrast to complete suppression at 1.0–10.0 u/kg observed when injected 60 min before the high food deprivation sessions.

A (three-between) × (one-within) group additive model analysis of variance [9] reveals a significant difference among response rates due to insulin dose: ($F(4,8) = 6.05$, $p<0.05$) and level of food deprivation ($F(1,2) = 10.13$, $p<0.05$). Pretreatment times alone had little overall effect on response rates ($F(1,2) = 4.63$, $p<0.20$). However, dose × level of food deprivation interactions were significant ($F(4,8) = 8.68$, $p<0.01$), as were dose × level of depriva-

tion x pretreatment time interaction effects ($F(4,8) = 11.11$, $p<0.01$).

DISCUSSION

These data partially confirm the Morgan and Morgan [10,11] findings that as pretreatment time increases to four hours the lever pressing rate-suppressant effect of insulin increases. However, the present experiment demonstrated this pretreatment time effect could be attenuated by decreasing the level of food deprivation. Booth and Brookover [2] also found that the operant rate-decreasing effects of a single dose of insulin were attenuated at lower food deprivation levels. The results of the present experiment suggest that the overall degree of response suppression is determined to a considerable extent by the food deprivation level; however, at high deprivation levels the pretreatment time becomes more crucial.

EXPERIMENT III: THE EFFECTS OF INSULIN ON FR 1 WATER REINFORCED LEVER PRESSING

Some reports have indicated that insulin treatment increases water consumption independent of food consumption [2, 3, 12]. However, no data have been published concerning the relation between water reinforced operant behavior and insulin administration. In the previous study it was found that insulin decreased FR 1 food reinforced behavior, the greater the dose and the longer the pretreatment time. The present study was undertaken to determine if insulin had similar effects on water reinforced behavior, thus clarifying the specificity of insulin's rate-suppressing effect.

METHOD

Animals

Four naive male albino Holtzman rats, nine months old at the beginning of this experiment, were used. During the experiment Rat C2 weighed 470 g; Rat C3, 530 g; Rat B1, 480 g and Rat C1, 490 g.

Apparatus

The apparatus was the same as in Experiment II except that the food magazine was disconnected and a Foringer water dipper was programmed to deliver 0.25 ml of tap water per lever press.

Procedure

The procedure was the same as Experiment II except that rats were 23 hr water deprived and conditioned to bar-press on a FR 1 water reinforcement schedule. Water was available only during the 50 min sessions and food was freely available only in the home cages. Thirty and 60 min pretreatment intervals were used. Rats C2 and B1 were injected first at the 30 min and then at the 60 min pretreatment time while Rat C3 was injected first at the 60 and then the 30 min interval. One rat, C1, exposed to alternating pretreatment times, died before completion of the final dose series.

RESULTS

All data were pooled into 30 and 60 min pretreatment groups for graphic presentation. However, the data for one animal that died (C1) after completion of only one complete pretreatment time dosage series were omitted from the statistical analysis.

The dose–mean lever press rate curves are shown in Fig. 3. Lever pressing following saline administration was negatively accelerated over the 50 min sessions. When injected 30 min before the session there was an overall dose-dependent response decrement characterized by an almost complete cessation of responding in the last 20 to 30 min of the insulin sessions at higher doses. At the lowest doses, 0.1 and 1.0 u/kg, there was very little change from the range of mean control rate of responding; Rats C2, C3 and C1 exhibited a 20 percent increase above mean control rates. At higher doses there was a slight decline for all animals. Two rats, C3 and C1, died following injection of 40 u/kg. When injected 60 min before the session, responding again decreased to near zero values early in the session as a function of dose, and the overall decline from mean control rate appeared to be even more pronounced, although this difference from the 30 min pretreatment dose effects was not significant ($F(1,2) = 1.11$, $p<0.20$). A (two-between) x (one-within) group additive model analysis of variance [9] reveals a nonsignificant difference among response rates due to insulin dose ($F(4,8) = 3.75$, $p<0.10$) or pretreatment times ($F(1,2) = 1.11$, $p<0.20$) although the trend was in the expected direction. Therefore, a further test was conducted to determine if the nonsignificant main linear dose effect was significant when changed to a log-linear distribution. A post-hoc contrast test [9] revealed a significant log-linear distribution for the dose effects ($F(1,8) = 29.2$, $p<0.05$), indicating that significant response suppression was apparent as a function of the log of the doses used.

DISCUSSION

Chronic water deprivation in rats also reduces free food intake; therefore the water deprived rats may have also been mildly food deprived in this experiment. Certainly the attenuated performance changes following insulin injections in these water deprived rats were generally similar to those found in Experiment II (B) when rats were only mildly food deprived (i.e., the low deprivation condition).

The similarity of these shallow dose-response functions may therefore be related to a protective or insulin-antagonizing function of prefeeding. It does not appear to be related to within-session food pellet ingestion since similar functions were found using water reinforcement, which is non-nutritive. It is interesting to note in this regard that oral glucose pretreatment specifically blocks insulin-induced eating [3,8].

Finally, two of the four rats died. Death occurred at 40 u/kg injected 30 min prior to water access. Thus, prefeeding may partially antagonize some of the effects of insulin but may not necessarily protect the organism from the lethal effects of high insulin doses.

EXPERIMENT IV: EFFECTS OF INSULIN OF VI 1 FOOD REINFORCED LEVER PRESSING

Under what would appear to be similar food deprivation conditions, similar dose effects were found on FR 1 food and water reinforcement schedules in Experiments II

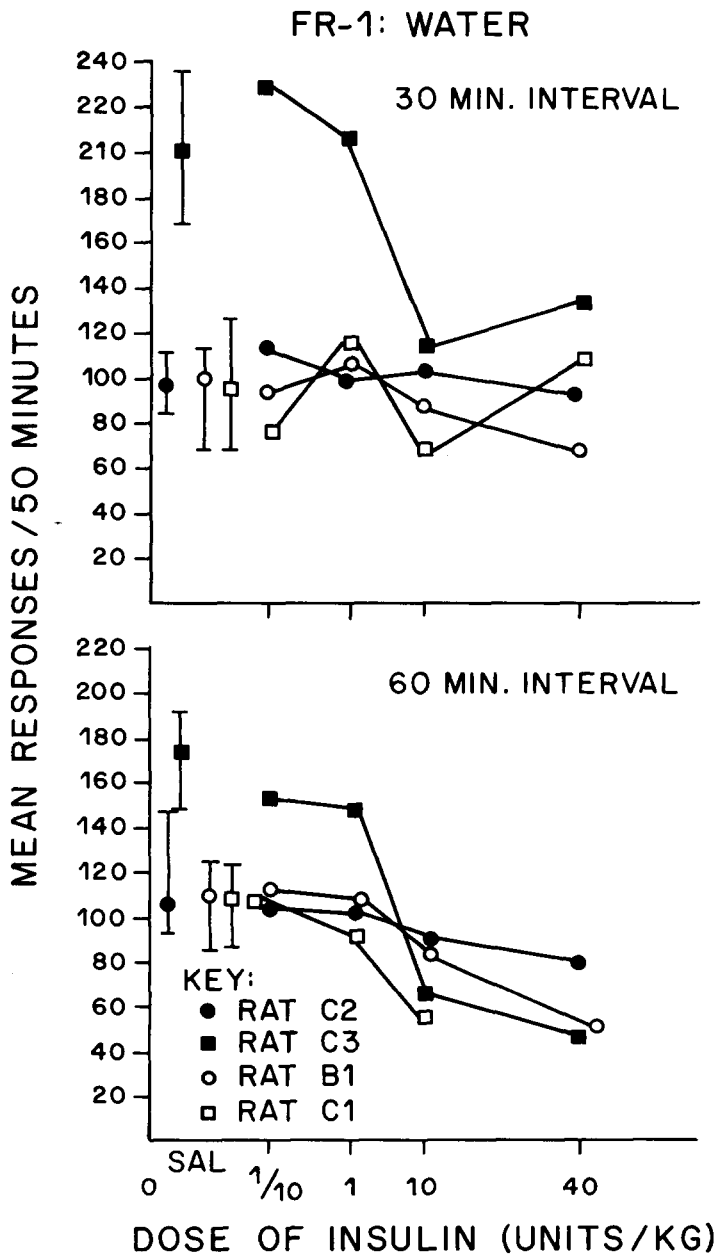

FIG. 3. Effects of regular insulin in doses of 0.1–40 u/kg on fixed ratio 1 water reinforced responding, as a function of pretreatment intervals. Baseline data for each subject are expressed as means (points) and ranges (vertical bars). Top graph presents data for 30 min pretreatment and bottom graph for 60 min pretreatment interval.

(B) and III. An explanation of the effects obtained in Experiments II (B) and III may be that the schedule of reinforcement (i.e. FR 1) is a more crucial determinant of the behavioral effect of insulin than is the type of reinforcer (i.e., food or water). Consistent insulin-induced rate increases were not seen in either of these studies nor in the modified FR 1 food reinforcement studies of Morgan and Morgan [10,11]. However, some investigators have noted insulin-induced rate increases using intermittent food reinforcement schedules [2,16]. Therefore, Experiment IV was conducted to evaluate the effects of insulin on intermittent schedule-controlled lever pressing.

In a variable interval 1 min (VI 1 min) reinforcement schedule food is available on the average of once a minute following the preceding reinforcement with the interval varying from reinforcement to reinforcement. This schedule has several important features. First, it holds the maximum number of pellets available per hour approximately constant; therefore a wide range of response rates will not change the overall reinforcement frequency (pro-

vided that responses are emitted with a mean value of approximately once per minute). This also minimizes satiation toward the end of the session, a problem which may have complicated other intermittent schedule studies using such low parameter values (FR 10 and FI 15 sec) that a large number of pellets were ingested in a very short time [2]. Second, steady-state responding on VI schedules is remarkably stable [6] thereby reducing intra- and inter-session variability. Finally VI schedule-controlled behavior has shown to be sensitive to changes in level of food deprivation [4,6], a variable which insulin is purported to influence [7].

METHOD

Animals

Five naive adult male Holtzman rats, 5 months old at the beginning of this experiment, were maintained at 85% of their free-feeding body weights: Rat I4 weighed 420 g; Rat A3, 420 g; Rat A4, 405 g; Rat I5, 425 g and Rat I6, 415 g.

Apparatus

The apparatus was the same as in Experiment II except that a Foringer tape-timer was added to the programming equipment for the VI 1 min schedule sessions.

Procedure

Following initial lever press training on a FR 1 schedule of food reinforcement for 60 min a day for two days, the schedule was shifted to a VI 20 sec reinforcement schedule. The VI 20 sec schedule was in effect for three to five days when the schedule was shifted to VI 40 sec for 10 days. Approximately two weeks after the beginning of training the VI 1 min schedule was introduced. Training on this schedule remained in effect for 60 days before drug treatments were initiated. The terminal session length was 100 min.

Water was available only in the home cage. Animals were fed to 85% of their free-feeding weights immediately following each session.

As in the previous studies, saline and insulin injections were given subcutaneously on alternate days. The time of injection was determined by the sequence of pretreatment times. The pretreatment times were, in order, 15, 60, 30 and 240 min. Within each pretreatment time the effects of six does levels – 0, 5, 10, 20, 40 and 60 u/kg – were explored. Additional dose levels ranging from 1/10 to 80 u/kg were also used at various pretreatment times in an attempt to find the optimal dose range for each subject. The order of dosage presentation was randomized across subjects. In general, subjects were given an initial dose of 10 or 20 u/kg and then the dose was varied depending on the effects of the initial dose, until the entire range had been sampled. The highest dose was defined as the dose at which complete suppression of lever pressing occurred or as 60 u/kg, if responding was not entirely suppressed under the particular treatment conditions. This dose was usually administered last in the series to avoid possible lethal effects of prolonged hypoglycemia. The series of doses was then usually replicated from lowest to highest dose for each animal.

RESULTS

Figure 4 shows the individual mean responses per 100 min for animals on a VI 1 schedule as a function of dose at various pretreatment times. Comparing values at 15 and 30 min, and 60 and 240 min intervals yield strikingly different curves. The rate decrement at 15 and 30 min times is generally asymptotic at 40 u/kg. By contrast the rate suppressing effect at 60 and 240 min is maximal (i.e., total suppression) at about 20 u/kg.

Individual probe doses of 1/10 to 5 u/kg failed to produce lever press rate-increases at any pretreatment time. A summary of the relation of the change from mean control rate as a function of representative low to moderate probe doses given at the four pretreatment intervals for each animal are shown in Fig. 5. For two rats, I4 and I6, there is a progressive decline in response rate the greater the pretreatment time. This effect was greatest at doses of 5 to 10 u/kg. For three remaining rats, A2, A3, and I5, the decline in overall rate was greatest at the 60 min pretreatment time while at low doses responding tended to return to control levels at the 240 min pretreatment time. Baseline VI 1 control response patterns were linear, with no instances of changes in local rates of responding. VI 1 response patterns following insulin were also linear across sessions, with minimal changes in local rates. Therefore, insulin reduced overall rate but not the local pattern of responding.

A (two-between) × (one-within) groups additive model analysis of variance [9] reveals a significance difference among response rates due to both insulin dose ($F(5,20) = 17.47$, $p<0.001$) and pretreatment time ($F(3,12) = 14.13$, $p<0.001$).

A post hoc contrast test on the dose × pretreatment time interaction shows a significant difference for a log linear dosage effect when the combined 15 and 30 min pretreatment time effects were contrasted against the combined 15 and 240 min pretreatment time effects ($F(1,60) = 5.89$, $p<0.025$). This indicates that the slopes for the combined 15 and 30 min pretreatment time effects are less than the slopes for the combined 60 and 240 min pretreatment time effects.

In summary, these data show a dose-dependent decrement in response rate, the decrement being about four times greater at the 60 and 240 min pretreatment times than at the 15 and 30 min pretreatment times.

DISCUSSION

The effects of insulin on VI 1 min schedule-controlled behavior were not qualitatively different from that of FR 1 food reinforced or water reinforced behavior. There was no reliable increase in response rate under any treatment condition. Instead response rate systematically decreased as a function of dose and pretreatment time. This suppression of operant lever pressing is not consistent with the hyperphagia reported for insulin in previous studies not involving an operant response [2, 3, 7]. Although our food reinforced animals were water deprived during the sessions, it seems unlikely that this lack of water availability accounts for the difference; insulin hyperphagia without polydipsia or before water intake has been reported [3].

It may also be argued that these results cannot be correlated with free-food access or hyperphagia studies because we did not first conduct a free-food experiment to gauge the effective dose range in these subjects for insulin hyperphagia. While this is true, it should be pointed out that insulin hyperphagia is purported to be a rather robust phe-

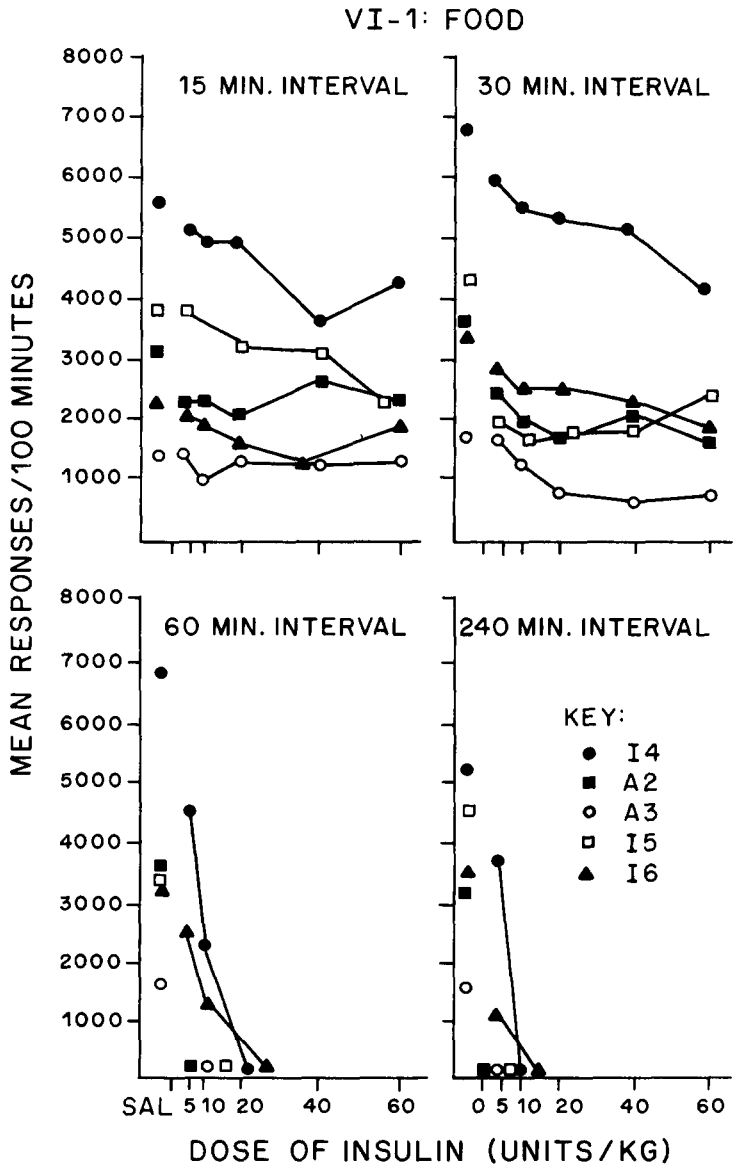

FIG. 4. Dose-response curves on the effects of regular insulin at 15, 30, 60 and 240 min pretreatment times, on variable interval 1 min food reinforcement performance.

nomenon, having been found in a variety of species, including man, across a wide dose range [2, 3, 7].

These results then do not support the notion that insulin selectively increases the effective food deprivation level as revealed by food reinforced operant behavior [7]. Such results are puzzling only if a unitary motivational mechanism is presumed to mediate the emission of the operant. Similar apparent inconsistencies have been found in the study of salt-induced and lesion-induced motivations. Sodium chloride injections enhance free (i.e., non-response contingent) water intake in rats across a wide concentration range; across this same range VI 1 water reinforced behavior showed a concentration-dependent decrease. FR 1 water reinforced behavior showed a slight increase at the lowest concentration but suppression at intermediate to high concentrations within this same polydipsia-inducing concentration range [17]. Hyperphagia following ventromedial hypothalamic lesions can also be abolished when food is made continent on emission of an operant [15]. Thus, although the results of specific motivational manipulations (e.g., food deprivation) and certain chemical or neurosurgical treatments may generate analogous effects using some measures (e.g., free food or water intake), their effects on simple deprivation-sensitive operants may be quite disparate. Either the mechanisms of action are not homologous for non-contingent and response-contingent access, or other deprivation-independent mechanisms become prepotent under treatment conditions which require emission of an operant.

In the case of insulin, either alternative is likely. Al-

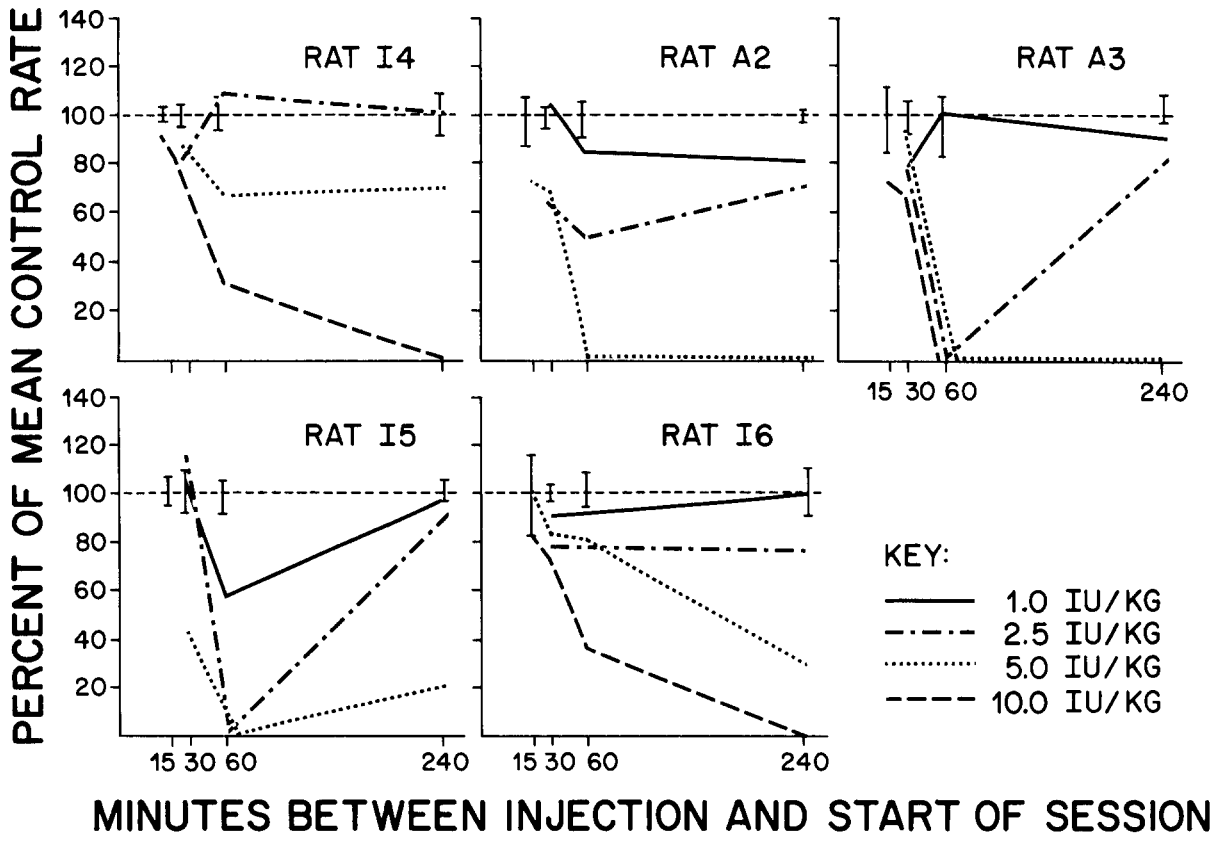

FIG. 5. Effects of pretreatment time and selected regular insulin doses on variable interval 1 min food reinforced performance expressed as percent of control rate. Baseline performance is indicated by dashed line parallel to the ordinate intersecting abscissa at 100%. Points indicate means and vertical bars represent ranges.

though the effects of insulin may resemble some of the effects of food deprivation, the evidence obtained in these studies suggests that the operant effects of insulin do not uniquely covary with the effects of food deprivation. Therefore, the behavioral mechanism of action of insulin does not appear to be simply related to an effect on the food deprivation level. More behavioral data will be needed to evaluate whether this is due to other independent mechanisms.

There is, however, some evidence that other deprivation-independent mechanisms are involved. At moderate doses and long pretreatment times (60 min) gross observation indicated that highly food deprived rats in Experiments I, II (A) and IV were awake but often flaccid and docile, even when handled. Responding was entirely suppressed under these conditions. The observed pattern is characteristic of insulin shock. Shock may certainly be considered incompatible with bar-pressing, but is not necessarily incompatible with the ingestion of free, response-independent food in the typical insulin hyperphagia situation. Thus, at moderate doses and long pretreatment times the responsible mechanism may be a non-specific physiological state which is incompatible with lever pressing, possibly due to direct muscular involvement.

This explanation would not account for the graded suppression at shorter (15–30 min) pretreatments; responding was much less suppressed in all behavioral experiments. Correlated data from Experiment I suggests that plasma glucose levels were falling during these pretreatment periods and animals were observed to be active and alert with no signs of flaccidity, ataxia or hyperreflexia.

These graded behavioral suppression effects of insulin at shorter pretreatments may be related to an interaction between feeding cycles and the elicidation of compensatory glucostatic mechanisms. At short pretreatment times the rapid fall in plasma glucose may be partially antagonized within a session by either the ingestion of food pellets in the conditioning chamber [Experiment II (A) and IV] or by prefeeding with [Experiment II (B)] or without [Experiment III] food available in the conditioning chamber. The continued graded response suppression may result from hypoglycemia *per se*, the elicitation of compensatory hormonal response systems such as epinephrine, ACTH or glucagon release [14] and/or from direct insulin activation of the ventromedial hypothalamic satiety center [5].

When allowed access to response-contingent food on either FR 1 or VI 1 min schedules responding was less suppressed when pretreatment intervals were 30 min or shorter. Again this suggests that food consumption before maximal hypoglycemia may partially ameliorate the rate suppressing effects of insulin in highly food deprived animals. Furthermore, when less food-deprived, the FR 1 food and FR 1 water reinforced behavioral preparations exhibited much less insulin-induced suppression, even at the 60

min pretreatment times.

The present series of studies indicate that under certain conditions either the dose, the level of food deprivation or the pretreatment time interval can influence the behavioral effects of insulin. Furthermore, at high food deprivation levels the schedule of reinforcement (i.e., FR 1 vs VI 1 min) made little difference in the rate-suppressant effect of insulin and at low food deprivation levels type of reinforcer (i.e., food vs water) made little difference in the degree of suppression on FR 1 schedules.

In the FR 1 food reinforcement study (Experiment II) it appears that access to a minimal amount of food may be necessary to counteract the rate suppressing effect of insulin at high food deprivation levels. Data obtained using the VI 1 min food reinforcement schedule further support this notion. They were restricted by the schedule from having access to food reinforcement more than once per minute on the average. This is in contrast to the FR 1 schedule which permits essentially unlimited response-contingent food access. In spite of this difference in reinforcement frequency between FR 1 and VI 1 min schedules both studies revealed similar dose-response functions. Therefore, reinforcement frequency over the range studied here appears to have little influence on differentially counteracting the insulin-induced rate suppression at high food deprivation levels.

Furthermore, level presssing maintained using FR 1 food and FR 1 water reinforcement schedules exhibited nearly identical dose-response curves at low food deprivation levels (the water reinforced preparations were not food deprived). The frequency of food reinforcement was zero for water reinforced lever pressing but essentially unlimited for the food reinforced rats. Therefore, it seems unlikely that frequency of food reinforcement *per se* is the primary determinant of the behavioral mode of action of insulin across the range of parameters studied here.

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