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Insulin-Induced Drinking: An Analysis of the Involvement of Renal Angiotensin II and Insulin-Induced Changes in Plasma Volume

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WALDBILLIG, R. J. AND T. J. BARTNESS. *Insulin-induced drinking: An analysis of the involvement of renal angiotensin II and insulin-induced changes in plasma volume.* PHARMACOL BIOCHEM BEHAV **28**(4) 447–452, 1987.—Male Long-Evans rats were used to investigate the potential hydrational mechanisms underlying insulin-induced drinking (IID). Plasma volume effects of insulin were assessed using both hematocrit and dye dilution procedures. Evidence is presented indicating that insulin produces a long-lasting and dose-dependent reduction in plasma volume. However, it does not appear that the drinking response is tightly tied to the reduced plasma volume because a 50% blockade of this effect does not reduce water intake. In addition, it does not appear that insulin-induced drinking is related to a release of renal angiotensin because IID is not blocked by nephrectomy. The mechanism underlying IID may be related to the activation of the recently described brain insulin receptor system.

Insulin Nephrectomy Drinking Baroreceptor Plasma volume Angiotensin

ALTHOUGH the majority of research on insulin has focused on its effects on food intake and glucose metabolism, there are now several reports indicating that insulin administration increases water intake [6, 14, 17, 20]. Previous work on the mechanism of insulin-induced drinking (IID) found that, although insulin injected animals were hypervolemic relative to their pre-injection baseline [4], the onset of IID is associated with an insulin-induced reduction of plasma volume [20]. Without taking a position on the role of endogenous insulin in naturally-occurring drinking, the work presented here further examined the hydrational basis of insulin effects on water intake.

This work began by determining whether the observed effect of insulin on plasma volume was idiosyncratic to the use of hematocrit measures. More specifically, the first project in this series used both dye dilution and hematocrit procedures to assess the effect of insulin on plasma volume. The second experiment in the series used nephrectomy procedures to determine whether renal renin angiotensin release was involved in IID. Finally, the third project determined whether IID was dependent upon other aspects of the plasma volume reduction (possibly mediated by putative baroreceptor responses). To accomplish this, an osmotically mediated expansion of plasma volume was used to off-set insulin-induced reductions in volume. The effect of this blockade on insulin-induced water intake was then determined.

EXPERIMENT 1

Early work examining the hydrational basis of IID [20] used hematocrit measures to assess the effects of insulin on plasma volume. This measure was selected over the dye dilution method because it allows rapid and repeated measures to be obtained from a single animal across several months of testing. Hematocrit procedures were also chosen because hematocrit and dye dilution estimates of total plasma volume are highly correlated [21]. This correlation is robust and is uninfluenced by either food or water deprivation [11]. Finally, the earlier work had selected the hematocrit measure because reviews concerned with the use of this technique in insulin administration experiments had concluded that there was no basis for its rejection [15].

Despite the above arguments, it must be acknowledged that the hematocrit measure represents the ratio of erythrocyte and plasma volume in the vasculature. Because of this, the use of this technique to assess plasma volume is valid only when the number of erythrocytes (RBCs) in circulation remains constant. With this potential complication in mind,

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it can be argued that previously reported effects of insulin on plasma volume actually represent an insulin-induced increase in the number of circulating red blood cells. Two mechanisms that could produce such an increase are: (1) an insulin-induced splenic contraction that forces red blood cells into the circulation and/or (2) an insulin-induced sympathetic discharge that flushes RBCs from the venous to the general circulation. In arguing against these effects, it has been pointed out that a splenically mediated increase in the hematocrit measure is unlikely because insulin administration does not alter splenic weight [20]. In addition, it has been pointed out that an insulin-induced adrenal-sympathetic discharge, flushing RBCs into the general circulation, is unlikely because insulin-induced changes in hematocrit are not blocked by adrenal demedullation [20].

Experiment I used two methods to further address the validity of using hematocrit to assess short-term changes in plasma volume. The first method examined whether there was a splenic influence on the hematocrit measure by determining whether splenectomy affects the magnitude of the insulin effect. The second procedure used to test the validity of the hematocrit method was to make within animal comparisons of hematocrit and dye dilution estimates of insulin-induced changes in plasma volume.

The results of this work indicate that: (1) insulin-induced changes in hematocrit are not the result of a splenic release of red blood cells, and (2) that insulin-induced reductions in plasma volume are observed when plasma volume changes are assessed with dye dilution procedures.

METHOD

Splenectomy

To determine the effect of splenectomy on insulin-induced changes in plasma volume, male Long-Evans rats (N=18) were individually housed and maintained (lights off from 2100 to 0730 hr) with ad lib access to food and water (body weight mean±SEM; 342.1±12.4 g). To determine hematocrit measures of plasma volume, duplicate blood samples (sample volume=100 μl) were obtained from the tip of the tail before and 60 minutes after intraperitoneal injections of insulin (20 U/kg) or insulin vehicle (NaCl 0.9%). The methods used for hematocrit determination have been previously described [20]. All intraperitoneal injections were equivolemic (0.1 ml/100 g). Previous work has shown that insulin-induced changes in hematocrit are not idiosyncratic to the use of blood samples taken from the tail [20]. Pre-injection blood samples were obtained within 90 seconds of removing the animal from its home cage. The animals were returned to their home cage without food and water for the interval between blood samples. Insulin and control injections were alternated across days and at least two days separated each injection. Each animal received four insulin and four control injections. After the baseline data had been collected, the animals were anesthetized with Nembutal (50 mg/kg) and either splenectomized or sham splenectomized. Following a one week recovery period, the effects of insulin on hematocrit were re-determined. All tests were conducted in the light phase of the photo cycle.

Dye Dilution

To determine whether insulin-induced reductions in plasma volume can be demonstrated with dye dilution methods, 14 male Long-Evans rats (mean±SEM; 356.2±7.3 g) were implanted with a unilateral, chronically indwelling, jugular catheter. After a two day post-surgery recovery period, the animals were transferred to a test chamber where the jugular catheter was attached to a 14 inch length of tubing, allowing remote intravenous infusions and blood sampling. After this connection had been made, the animals were habituated to the chamber for eight hours. At the conclusion of the habituation interval, food and water were removed and the animals were infused with 0.2 ml of 1.0% Evans Blue dye. Thrity minutes later a pre-injection blood sample was drawn for optical density and hematocrit measurements of plasma volume. Immediately after this blood sample had been obtained the animals were injected with insulin (20 U/kg) or insulin vehicle. Post-injection blood samples were taken 30, 60, and 90 minutes later. The volume of blood taken for the samples was replaced with isotonic saline.

RESULTS

Splenectomy

In the pre-splenectomy condition it was found that, relative to the pre-injection baseline, control procedures *per se* produced a statistically significant reduction (1.9%±0.10) in hematocrit, $t(17)=5.63$, $p<0.01$. This decrease in hematocrit is interpreted to indicate an increase in plasma volume. In previous reports, this effect has been referred to as "handling-induced hypervolemia" [4]. The baseline hematocrit on which this increase in volume was observed was 53.8±0.9%. Also, in the pre-splenectomy condition, it was found that insulin administration significantly reduced the increase in plasma volume produced by the injection procedure *per se*. The magnitude of the insulin effect was a statistically significant 1.7±0.32% reduction in plasma volume, $t(17)=8.43$, $p<0.001$. The effect of insulin is calculated as the difference between the plasma volume effects of insulin and control injections. This demonstration of an insulin-induced reduction and handling-induced increase in plasma volume replicates earlier findings [4,10].

In the post-splenectomy condition, insulin and control injections continue to produce significant alterations in plasma volume, $t(7)>6.41$, $p<0.01$. The magnitude of the insulin effects in the splenectomy and sham-splenectomy group were 2.1±0.41% and 1.91±0.12% respectively. This difference is not statistically significant ($p>0.05$). Indeed, across all conditions (i.e., pre-splenectomy, sham splenectomy and splenectomy) the insulin effect was not statistically different ($p>0.05$).

Dye Dilution

Regardless of the technique used to assess changes in plasma volume (dye dilution or hematocrit), insulin produced a statistically significant reduction in plasma volume, $t(6)>4.35$, $p<0.01$. The dye dilution measurements specifically indicated that insulin reduced plasma volume by 4.5, 6.6, and 6.9% at the three successive (30, 60 and 90 minute) measurement points. Hematocrit measures at these same time points also revealed insulin-induced plasma volume reductions of 1.2, 2.3, and 2.8%, $t(6)>4.59$, $p<0.01$. Because the percent changes in hematocrit are smaller than the percent change obtained with the dye dilution procedure, it may be that hematocrit measures underestimate the magnitude of insulin's effects on plasma volume.

The agreement between hematocrit and dye dilution methods in assessing the effects of insulin on plasma volume was also seen when these methods were used to assess the

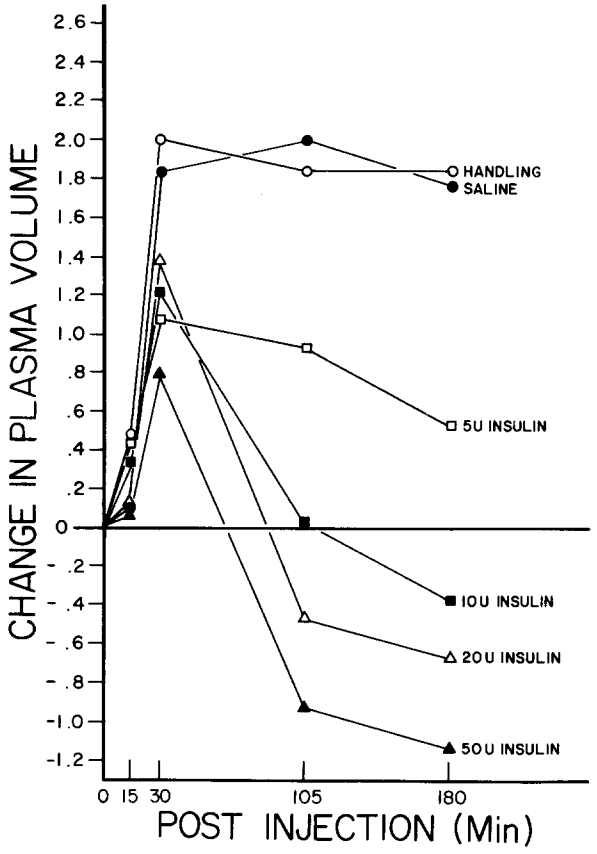

FIG. 1. Effect of insulin and sham injections on plasma volume. The mean pre-injection hematocrit was 52.7±7%.

effects of control procedures. Here both methods indicate that control procedures produce a significant increase in plasma volume, $t(5)>3.59$, $p<0.01$. However, in contrast to earlier work where the increased volume was rapid (latency<30 min), here the increase in plasma volume (hematocrit, 2.37%; dye dilution, 8.3%) became statistically significant only at the 90 minute measurement point. The lack of a significant plasma volume expansion at the 30 and 60 minute measurement point may be related to the diminished handling of the animal with the remote infusion method. An overall examination of the data indicates that dye dilution and hematocrit measures of insulin and control effects on plasma volume are significantly correlated ($r=0.76$, $p<0.05$).

DISCUSSION

These data indicate that insulin-induced changes in hematocrit are not artifactually related to splenic contractions that increase RBC number. Additionally, it was shown that insulin-induced reductions in plasma volume are not idiosyncratic to the use of the hematocrit measure because similar reductions are observed when plasma volume changes are assessed with the dye dilution method.

EXPERIMENT 2

The previous experiment validated the use of hematocrit to assess insulin effects on plasma volume. Because of its covenience, Experiment 2 used hematocrit to examine the dose-response characteristics and time-course of insulin effects on plasma volume. In addition, this experiment, by including a sham-injection condition (insertion of needle without fluid delivery), further assessed the basis of the increase in plasma volume seen following control injections.

METHOD

Six male Long-Evans rats (386±20.7 g) were individually housed with ad lib access to Purina laboratory chow and tap water. To determine the effects of insulin on plasma volume, blood samples were taken from the tip of the tail (duplicate measures each of 100 μl volume) immediately before and 15, 30, 105, or 180 minutes after intraperitoneal injections of insulin (5, 10, 20, 50 U/kg) or saline (0.9%). Except for the sham injections (zero volume), all injections had a constant volume (0.1 ml/100 g body weight). The order of interval testing was varied in a pseudorandom fashion (30, 15, 180, and 105). Two days separated all injections and the injection type (i.e., sham, insulin, and vehicle) was pseudorandomly varied across tests.

RESULTS

Figure 1 shows, as a function of time and dose, the effects of insulin, control, and sham injections on plasma volume. Sham and control injections produce an equivalent increase in plasma volume that becomes asymptotic within 30 minutes and lasts for at least three hours. In contrast to this, insulin injections produce a dose-dependent and long-lasting reduction in plasma volume. The insulin-induced reductions in plasma volume occur within 30 min and increase in their magnitude over the three hour interval. The magnitude of the insulin-induced decrease in plasma volume (absolute difference between the effect of insulin and control injections) increased with both post-injection time and dose, Two Way ANOVA, Dose and Time $Fs(3,15)>7.94$, $p<0.01$. The Dose × Time interaction was statistically significant, $F(3,25)=3.41$, $p<0.05$. This reduction in plasma volume at the 30 and 50 U/kg doses was significantly greater than the reduction produced by the 5 U/kg dose (Duncan's New Multiple Range [DNMR], $ps<0.05$). Finally the magnitude of the insulin effect at 105 and 180 minutes was greater than the 15 minute reduction (DNMR, $p<0.05$).

DISCUSSION

These results indicate that insulin produces a dose-dependent and long-lasting reduction in plasma volume. However, the time course of the insulin and "handling" effect is such that, at the 30 minute point (the typical point for the onset of IID tests), insulin-injected animals are hypervolemic relative to their pre-injection baseline. Because *hypo*volemia (not hypervolemia) is associated with the onset of drinking, this finding may indicate that the insulin-induced reduction in plasma volume is not causally related to IID. A contrasting analysis could focus on the likely possibility that the onset of the insulin effect is delayed relative to the handling effect. As a result, insulin injected animals would have plasma volume levels that would expand and then contract. The downward trend in plasma volume *per se* may act as the critical signal to initiate water intake. Unfortunately the data required to evaluate this hypothesis is unavailable. An additional finding of this work was that control and sham injections produce equivalent increases in plasma volume. This indicates that the expansion in volume is not related to the actual volume being delivered during the control injections (0.1 ml/100 g).

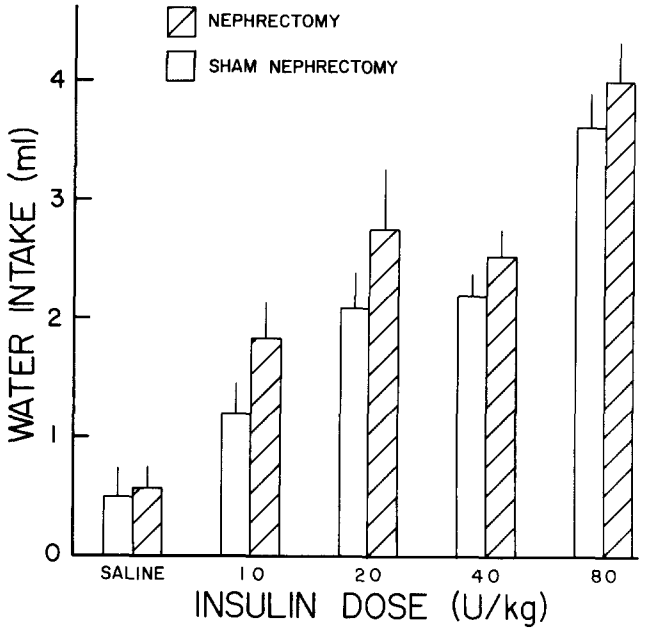

FIG. 2. Effect of nephrectomy and sham nephrectomy on the water intake of animals intraperitoneally injected with various doses of insulin.

Of course, the mechanism by which insulin reduces plasma volume remains to be determined. In this context however, we have found the insulin effect is enhanced in nephrectomized animals (unpublished data). Because of this, a mechanism involving insulin-induced diuresis seems unlikely. There is some evidence that insulin produces a translocation of albumin from the vasculature of juvenile diabetic humans [9]. Possibly, a similar effect occurs in non-diabetic rats and the loss of vascular fluid is secondary to an efflux of albumin from the vasculature. An additional insulin effect that could produce a loss of vascular fluid is an insulin-related increase in the secretion of gastric fluids [8].

EXPERIMENT 3

Previous work [20] and Experiment 2 indicate that, relative to a pre-injection baseline, insulin injected animals are hypervolemic at the onset of IID tests. In spite of this, there is some possibility that in the critical minutes preceding the drinking test, plasma volume is decreasing from these elevated levels. This downward trend could increase water intake secondary to the activation of the renal renin angiotensin system [7,12]. In addition, insulin may directly activate the renal angiotensin system. To test the possibility that IID is related to an increase in angiotensin II, the present experiment determined whether IID was affected by nephrectomy.

METHOD

Eighty-nine Long-Evans male rats (357±39 g) were housed individually and maintained with ad lib access to Purina laboratory chow and water. Using ether anesthesia, animals were either bilaterally nephrectomized or sham nephrectomized. The nephrectomy procedure was nearly bloodless and involved placing a double ligature on the renal artery and vein, cutting between the ligatures, and removing the kidney. The procedure for the sham nephrectomy included exposing the kidney and lightly probing the surrounding fat pad. Half the animals in each dosage group were nephrectomized, the remaining animals were sham nephrectomized. Following a two hour post-surgery recovery period, food and water were removed from the home cage, and the animals were injected intraperitoneally with regular insulin (10 U/kg, N=14; 20 U/kg, N=20; 40 U/kg, N=17; 80 U/kg, N=24) or 0.9% saline (N=14). Thirty minutes later the animals were given 60 min access to water but not food. The drinking test was conducted in the midportion of the 12-12 photoperiod light cycle and intake was measured directly from a graduated drinking cylinder to the nearest 0.1 ml.

RESULTS

Figure 2 shows the magnitude of IID following sham and complete nephrectomy. Nephrectomy did not block the capacity of insulin to elicit drinking. Insulin produced a dose-dependent increase in water intake in both groups. Figure 2 shows that nephrectomized animals drank more than the sham operated controls. Only at the 10 U/kg dose was this increased intake statistically significant. This resulted in a Dose × Surgery interaction, $F(4,79)=6.43$, and the DNMR test indicated the probability associated with the 10 U dose was $p<0.05$. At the doses of 20 and 40 U/kg this difference between the sham and completely nephrectomized animals only approached statistical significance ($p<0.10$).

DISCUSSION

The results of this work indicate that IID does not depend on the release of renal renin angiotensin [7] since insulin-induced drinking persists when the source of renal angiotensin has been removed. In fact, nephrectomized animals drink slightly more than the controls. We have found (unpublished observations) that nephrectomized rats have elevated levels of exogenous insulin and it is possible that the tendency to drink more water is related to this effect. The observation of increased insulin levels in nephrectomized animals fits with the finding that, in rats, the kidney is an important site of insulin degradation [10,16]. The increased bio-availability of insulin in nephrectomized rats would also account for our finding that nephrectomy significantly enhances the insulin-induced reduction in plasma volume (unpublished observations).

EXPERIMENT 4

The above work indicates that IID is not the result of insulin directly or indirectly releasing renal renin angiotensin [12]. However, other aspects of the reduction in plasma volume (e.g., a baroreceptor response) [23], may be causally related to the increased water intake. If this were the case, then one would expect that a blockade of the plasma volume reduction would prevent or reduce IID. To test this hypothesis, Experiment 4 uncoupled the effects of insulin administration *per se* from its effects on plasma volume. The rationale for this experiment was to block or off-set the insulin-induced reduction in plasma volume by producing an osmotically-mediated increase in vascular fluid. If IID is independent of the reduction in plasma volume, drinking should persist despite the prevention of this effect. The osmotically-mediated increase in plasma volume was produced by increasing the salinity of the insulin vehicle.

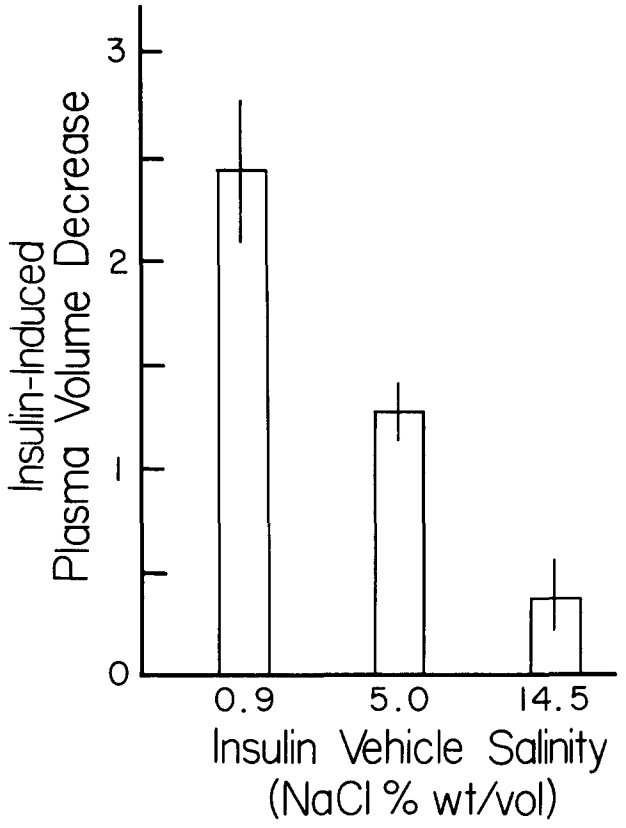

FIG. 3. Effect of varying the salinity of the insulin vehicle on the magnitude of the insulin-induced (50 U/kg) efflux of vascular fluid. Efflux=the absolute difference in the change in hematocrit plasma volume measures produced by intraperitoneal insulin and saline injections.

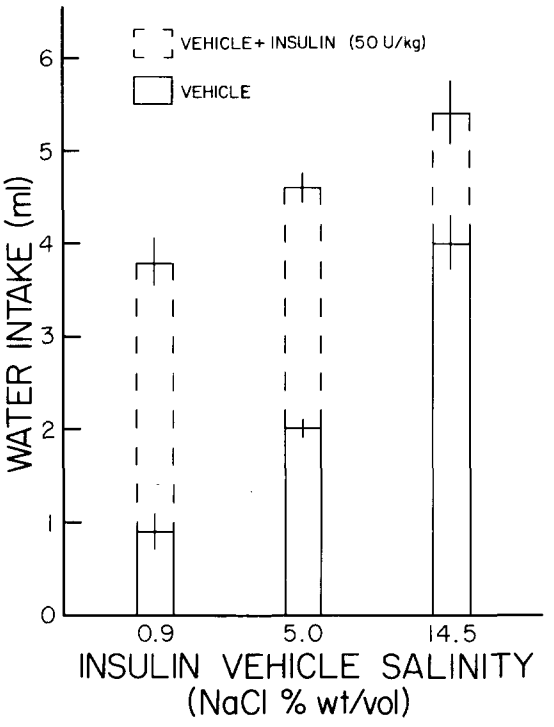

FIG. 4. Water intake of animals injected with insulin in vehicles of various salinity or vehicles alone.

METHOD

Ten male Long-Evans (body weight=284±0.9 g) rats were individually housed and maintained with ad lib access to Purina laboratory chow and tap water. The same group of animals was used to determine the effect of vehicle salinity on: (1) insulin-induced drinking, (2) NaCl-induced drinking, and (3) plasma volume.

In the IID test, animals were injected intraperitoneally with insulin (50 U/kg) and half an hour later given 30 min access to water, but not food. Food and water were not available in the interval between the insulin injection and the onset of the drinking test. To determine the effect of osmotically blocking the insulin-induced efflux of vascular water, insulin vehicle salinity was varied across IID tests. Vehicle salinity was either 0.9, 5.0, or 14.5% NaCl. All injections were equivolemic (0.1 ml/100 g body weight).

The effectiveness of vehicle salinity in blocking the insulin-induced reductions in plasma volume was determined in a separate series of tests where tail vein blood samples were obtained before and 30 min after the insulin injections. Food and water were not available in the interval between the blood samplings. Hematocrit methods were used to assess changes in plasma volume. All blood samples were processed in duplicate.

Separate drinking tests were conducted to determine the effect of vehicle salinity *per se* on water intake. In this work the IID protocol was used. In both the insulin- and vehicle-induced drinking tests, each animal was tested with each level of vehicle salinity five times. All drinking tests were separated from each other by at least two days and water intake was measured to the nearest 0.1 ml.

RESULTS

Figure 3 shows the magnitude of insulin-induced reductions in plasma volume. As previously noted, the insulin-induced reduction in plasma volume is the absolute difference between the plasma volume effects of insulin and control injections. Insulin in its normal vehicle (0.9%) reduced plasma volume by 2.49%. In addition, as the vehicle salinity was increased, the plasma volume effect of insulin was reduced. Vehicle salinity produced a significant blockade of the insulin effect and the magnitude of this blockade was progressively greater with successive increases in salinity, One-Way ANOVA; $F(2,18)=60.39$, DNMR $p<0.01$.

Figure 4 shows the water intake produced by injections of hypertonic vehicles alone or in combination with insulin (50 U/kg). The addition of insulin to vehicles produced a level of drinking that was greater than that produced by vehicle alone, Two-Way ANOVA; Injection type × Vehicle concentration; $F(1,9)=25.28$, DNMR $p<0.01$. Despite the fact that the 5.0% vehicle salinity blocked 50% of the insulin-induced plasma volume reduction (see Fig. 3), the magnitude of the insulin component of drinking was virtually unaffected (see Fig. 4). In contrast to the lack of an effect of the 50.0% blockade, the insulin component of drinking was much reduced when the vehicle NaCl conentration was increased to 14.5%. Recall that this level of vehicle salinity nearly totally blocked the efflux of vascular fluid (see Fig. 3).

DISCUSSION

Insulin-induced drinking persisted virtually undiminished despite a 50.0% blockade of the efflux of vascular water. This suggests that the drinking response is not tightly dependent upon the insulin-induced changes in plasma volume. More support for this view is the finding that the nearly total blockade of insulin-induced reduction in plasma volume (by the 14.5% vehicle) still allowed significant levels of insulin-induced drinking. The converse interpretation of the 14.5% data is that drinking is substantially suppressed when the insulin-induced efflux is blocked. It should be noted, however, that the suppression of intake at the higher levels of vehicle salinity may be secondary to debilitating effects of the hypertonic vehicles. However, we have found that the disruption of behavior by intraperitoneal hypertonic saline injections is rather short-lived [5]. Despite this, it remains to be unambiguously determined whether a complete blockade of the insulin-induced plasma volume reduction affects IID.

GENERAL DISCUSSION

The data presented here indicates that insulin produces a long-lasting and dose-dependent reduction in plasma volume. However, insulin-induced drinking does not appear to depend upon a release of renal renin angiotensin [7]. The finding that the insulin-induced efflux of vascular water can be reduced to half its normal magnitude without an appreciable effect on the magnitude of IID also appears to indicate that water intake is not tightly tied to the magnitude of the plasma volume reduction.

Another mechanism by which insulin could increase water intake is by producing plasma hyperosmolarity. However, previous work in this laboratory has shown that, depending upon dose, insulin has either no effect on plasma osmolality or produces a hypo-osmolality [20]. The hypo-osmotic effect of insulin may be related to an insulin-induced hypokalemia (for review see [13,22]).

From the data presented here it appears that the pharmacological effects of insulin may be responsible for IID. One such effect is a change in brain hydration produced by elevated brain sodium levels [1–3]. Another possibility is that IID is the result of blood-borne insulin that activates circumventricular [18,19] neural circuits that subserve some forms of drinking.

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