Naloxone Decreases Centrally Induced Hyperglycemia in Dogs

Evidence for an Opioid Role in Glucose Homeostasis

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SUMMARY

Intracerebroventricular (ICV) instillation of morphine and β-endorphin causes centrally induced hyperglycemia. Locally active, endogenous opioids in the central nervous system may, therefore, also be involved in the elevation of blood sugar. This possibility was tested by examining the glucoregulatory response to central glucoprivation induced by ICV administration of 2-deoxy-D-glucose (2DG) in dogs. Administration of 2DG resulted in a rise in plasma glucose and immunoreactive glucagon (IRG) of 108 ± 19 mg/dl and 70 ± 20 pg/ml, respectively. These changes were attenuated by the simultaneous central infusion of the opiate antagonist naloxone: plasma glucose levels increased by 77 ± 14 mg/dl and IRG by 43 ± 3 pg/ml, both significantly different from the effect of 2DG alone ($P < 0.05$–0.01). These findings suggest that opiate receptors participate in the counterregulatory response to central glucoprivation. They also provide a mechanism by which endogenous opioid peptides may play a role in the central regulation of glucose homeostasis.

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The central nervous system exerts a controlling influence on the maintenance of glucose homeostasis.[1] Thus, a decrease in glucose available for metabolic use by the brain in vivo stimulates counterregulatory responses that result in a rise in blood glucose level[2–6] and a restoration of central glucose delivery. Considerable attention has been focused on the effector mechanisms responsible for restoring glucose levels, but much less is known about the afferent pathways involved. The link between the central sensing of a deficit in glucose availability, and the compensatory rise in plasma glucose, has been examined in this report.

Evidence exists for a central glucoreceptor[7,8] sensitive to changes in blood glucose concentrations. Glucose-sensitive areas[9,10] of the brain, which appear to detect and initiate responses to glucoprivation, have been defined. The process responsible for this response is presumably dependent on release of appropriate neurotransmitters. Pharmacologic studies have identified a number of potential neurotransmitters that cause hyperglycemia[11–13] by central mechanisms; however, a role in mediating physiologically appropriate responses in states of glucose need has not yet been defined for these substances. One of these neurotransmitters is β-endorphin,[12] which, like morphine,[14–17] gives rise to centrally induced hyperglycemia that is reversed by an opiate antagonist. This property of the opiate antagonist, naloxone, was used to test the possibility that the endogenous opioids might take part in the hyperglycemic response to glucose deprivation. If indeed this was so, naloxone would be expected to diminish the hyperglycemic response to a physiologic stimulus such as glucoprivation. This possibility was examined using 2-deoxy-D-glucose (2DG), a glucose analogue that competitively inhibits glucose utilization.[18,19]

MATERIALS AND METHODS

Experiments were performed in eight mongrel dogs, weighing about 20 kg, in which indwelling catheters were placed in the lateral cerebral ventricle according to a previously described technique.[20] We chose to use the central administration of 2DG to examine selectively the neural pathway that mediates glucose counterregulation as expressed by changes in plasma glucose and immunoreactive glucagon (IRG).

Catheter placement. A burr-hole was drilled in the skull of the dogs, and a teflon catheter was then inserted into the left lateral cerebral ventricle under sterile conditions, as described previously.[20] Satisfactory placement was followed by fixation of the catheter to the skull surface using dental acrylic. Silastic tubing, previously attached to the teflon cath-

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eter, was led through a subcutaneous tunnel to the back of the neck where it was connected to a rubber injection port, also buried subcutaneously. This allowed subsequent percutaneous access to the catheter system under sterile conditions. The position of the catheter in the cerebral ventricle was determined at postmortem by injecting a dilute solution of dental acrylic into the catheter system, and allowing it to harden into a cast. Only animals in which a cast could confirm the position of the catheter are presented.

Experimental protocol. Experiments began 1 wk after surgery, and were carried out at intervals of not less than 1 wk thereafter, in random order. After an overnight fast, a catheter was placed in a foreleg vein for the purpose of blood sampling. Blood was drawn every 10–20 min. A baseline period of 20 min preceded the experimental infusion and samples were drawn for a further 120 min. 2-Deoxy-D-glucose (2DG), 37.5 mg/kg (Sigma), was infused into the lateral cerebral ventricle at a rate of 0.5 ml/min over a period of 15 min. In separate experiments, the same dose of 2DG was infused together with the opiate antagonist, naloxone (Endo), 5 mg, over 15 min. The naloxone infusion was continued for another 25 min at 0.04 ml/min, delivering another 5 mg. Dogs infused with 2DG alone continued to receive normal saline instead. Behavioral effects noted with the administration of 2DG were somnolence, salivation, and in some animals, vomiting. Metabolic responses are not affected by these changes,[20] nor was there an effect of naloxone on the appearance of side effects.

Analytical methods. Blood samples were collected into chilled tubes containing EDTA (1.2 mg/ml) and Trasylol (500 U/ml) and separated within 30 min. An aliquot of plasma was taken for glucose measurement (Yellow Springs Autoanalyzer) and the rest frozen at −20°C until glucagon radioimmunoassay.[20]

Results are expressed as mean ± SEM. In 3 of 8 dogs, either 2DG or 2DG plus naloxone was infused on more than one occasion, also in random order. No evidence for a tachyphylactic effect of repeated 2DG was observed under these conditions. For calculation of data in these animals, the results at each time point in similar experiments were averaged. Results were analyzed by the two-tailed Student's *t* test for paired samples.

RESULTS

Plasma glucose response. Our results demonstrate that 2DG caused a rise in plasma glucose concentrations of 108 ± 19 mg/dl from a baseline of 102 ± 5 mg/dl at 80 min ($P < 0.001$) (Figure 1); the plasma glucose remained elevated at 100 ± 18 mg/dl above baseline at 120 min ($P < 0.001$). These results are similar to those previously observed using the same model.[5,20] In contrast, in the presence of naloxone, the rise in glucose was attenuated. Baseline glucose concentrations, 100 ± 5 mg/dl, rose by 77 ± 14 mg/dl at 100 min, significantly different from the rise after 2DG alone at 60 ($P < 0.05$), 70, 80 ($P < 0.025$), and 90 min ($P < 0.05$). The overall decrease (area under the curve) induced by naloxone in the counterregulatory response to glucoprivation was 23.4%, such that approximately one-quarter of this glucose response may be attributable to mediation by endogenous opioid peptides.

Plasma IRG response. The possible mechanisms for the diminished glucose response were also examined. Plasma IRG, which rises after 2DG administration, may play some role in the ensuing hyperglycemia, and therefore was measured in the same experiments (Figure 1). IRG levels rose by 70 ± 20 pg/ml ($P < 0.01$) above a baseline of 46 ± 5 pg/ml after 2DG alone. When naloxone was also infused, the increase in IRG was diminished. IRG rose from 43 ± 3 pg/ml by 31 ± 10 pg/ml only, significantly different from the effects of 2DG alone at 20 ($P < 0.01$); 30 ($P < 0.02$); 40, 50 ($P < 0.05$), and 60 min ($P < 0.02$). The peak increase of IRG concentrations was also delayed (70 min versus 51 min, $P < 0.05$, by paired analysis of peaks in each experiment). This difference in glucagon output by the endocrine pancreas may account, in part, for the naloxone-induced attenuation of the rise in plasma glucose. However, the contribution of glucagon cannot be quantitated in the absence of measurements of catecholamines, which independently may cause hyperglycemia.

DISCUSSION

The results of these experiments appear to confirm the hypothesis that naloxone will reduce 2DG-induced hypergly-

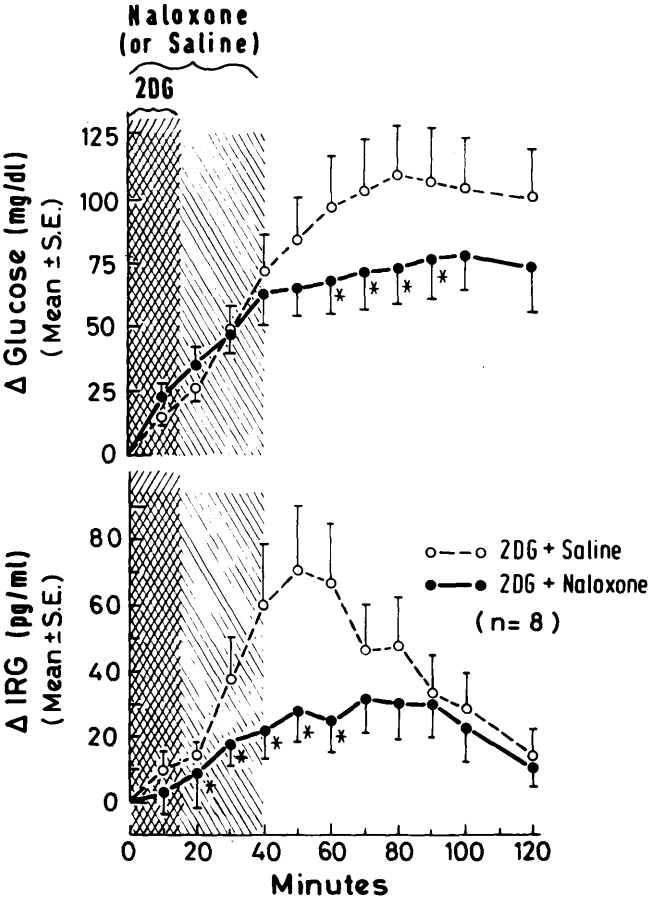

FIGURE 1. Comparison of the effects of intracerebroventricular 2-deoxy-D-glucose (2DG) with or without naloxone upon plasma glucose and immunoreactive glucagon (IRG) concentrations in normal conscious dogs (mean ± SEM). N = 8. Asterisks refer to statistically significant differences ($P < 0.05$–0.01) between the responses to 2DG alone and 2DG plus naloxone. Baseline levels of plasma glucose and IRG were: 2DG, 103 ± 5 mg/dl and 46 ± 5 pg/ml; 2DG + naloxone, 100 ± 5 mg/dl and 43 ± 3 pg/ml, respectively.

cemia. Additional evidence that supports these findings are some interesting parallels between the hyperglycemia observed after 2DG and that which occurs with morphine administration: (1) both 2DG[2-5] and morphine[14-17] are powerful inducers of hyperglycemia, which is prevented by adrenalectomy, confirming common dependence upon adrenergic pathways; (2) their probable sites of action in the hypothalamus and brainstem are similar;[9,10,17,21] (3) indeed, these are areas in which opioid peptides[22] and opiate receptors[23] are found; and (4) both 2DG,[9,24] and morphine-induced hyperglycemia[16] are suppressed by barbiturate anesthesia. These similarities support the possibility that after glucoprivation due to 2DG, the ensuing hyperglycemia may be mediated by a mechanism involving, to some extent, the activation of specific receptors by endogenous opioid ligands. Further experimental evidence implicating opiate receptors in 2DG-hyperglycemia is a preliminary finding suggesting that the rise in blood glucose is also reduced after repeated morphine injections in dogs (data not shown). The latter produces tolerance to the hyperglycemic effects of the opiates,[25] and thus may reduce the sensitivity of central opiate receptors to endogenous as well as exogenous opiates.

Previous studies examining the effect of naloxone upon basal[28] or stimulated glucoregulatory responses[26] have failed to demonstrate a significant effect of the opiate antagonist. For this reason, we examined the glucose responses during stimulated conditions (glucoprivation) when endogenous opiates, if involved, were most likely to be maximally active in causing hyperglycemia, and thus the blocking effects of naloxone more prominent; second, isolating the central mechanism of glucose counterregulation (ICV instillation) we excluded the possible confounding effects of other noncentral responses to glucose deprivation,[19] present in studies[26] in which generalized hypoglycemia was the tool employed to study this question. However, since the effect of naloxone under basal conditions was not examined in this study, we cannot assess whether maintenance of fasting blood glucose levels is dependent on central opiate mechanisms. This requires further investigation.

It has previously been suggested that opioid peptides may play a role in the maintenance,[27] as well as the abnormalities,[28-31] of glucose homeostasis. These studies provide evidence for a role of endogenous opioid mechanisms in the central control of circulating glucose by the central nervous system. They also provide further information about the intermediary pathways of central glucose counterregulation.

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