Letters to the Editor

Insulin Reduction of Cerebral Infarction

To the Editor: We commend Hamilton, et al. (Hamilton MG, Tranmer BI, Auer RN: Insulin reduction of cerebral infarction due to transient focal ischemia. J Neurosurg 82:262–268, February, 1995) for their careful investigation of independent effects of insulin and hypoglycemia on infarct size in a rat model after 2 hours of focal cerebral ischemia. They demonstrated significant reductions in infarct size in animals rendered slightly hypoglycemic with insulin whereas insulin administration alone, without the hypoglycemia, had no such effect. This finding of an ameliorative effect of hypoglycemia on infarct size conflicts with the results from our recently published work which demonstrates that slight hypoglycemia of the same magnitude in the cat aggravates rather than ameliorates the effects of focal ischemia: enlarging infarcts in survivors and increasing mortality compared to normoglycemia in permanent and 8-hour temporary middle cerebral artery (MCA) occlusion. Because a variety of mechanisms operate to define ischemic stroke outcome, it may be useful to compare similarities and differences in the two experimental situations in the hope of understanding why these observed differences in outcome occurred.

Both studies investigated the effects of proximal MCA occlusion: the influence of level of glycaemia on outcome and assessed infarct size morphometrically after long-term survival. Both studies also closely controlled a wide range of physiological variables that might affect outcome. The studies differed principally in the animal species used (rat vs. cat), ischemia duration (2 vs. 8 hours of temporary and permanent MCA occlusion), mean arterial blood pressure during ischemia (reduced to 60 vs. 125 mm Hg), type of anesthesia (halothane/nitrous oxide vs. pentobarbital) and animal death from brain herniation (absent vs. 0%–40% of groups depending on glycaemia and duration of ischemia).

The use of a rat model by Hamilton, et al., and the reduction of blood pressure during ischemia to 60 mm Hg may account for many of the differences in outcome observed. The cat brain generally exhibits good collateral vessels between the main cerebral supply territories contributing to a larger infarct size variability from MCA occlusion than in the rat. The duration of reversibility from MCA occlusion is markedly longer in cats (4 hours) than in rats (45 minutes), suggesting that the anastomotic links between the rat’s cerebral arteries are less well developed. Marked reductions in blood pressure likely aggravate this situation still further, reducing the opportunity for alternate tissue blood perfusion to compensate for loss of primary blood flow. This combination of limited collateral blood vessels and reduced blood pressure may produce a close to “end-artery situation” in these rats. The effect of markedly reduced collateral blood flow may then approach anoxia of the tissue, a state in which hypoglycemia indeed protects the brain.

Studies of ischemic cerebral blood flow (CBF) using the hydrogen clearance technique in our cat model demonstrate that improvements in collateral blood flow actually take place. Occlusion of the proximal MCA in normo- and hyperglycemic cats similarly reduces the CBF through its drainage territory by approximately 70% of control flow at 30 minutes after occlusion. The normoglycemic group’s CBF significantly ameliorates to approximately 40% reductions by 4 and 8 hours of occlusion. The marked improvement in ischemic CBF present in normoglycemic animals did not take place in the hyperglycemic cats exhibiting no significant change over time. Although information regarding the ischemic CBF effects of hypoglycemia is not available, a similar lack of improvement in collateral blood flow to that which occurred in the hyperglycemic animals might account for hypoglycemic animals’ increased morbidity and mortality following both temporary and permanent MCA occlusion.

Thus, when the ischemic CBF reduction is extreme, achieved by induced hypotension in a near end-artery situation, the expected metabolic protection of hypoglycemia may appear even in focal cerebral ischemia. However, in another animal species, which has a more abundant collateral blood flow and in the presence of a normal blood pressure, the adverse effects of hypoglycemia override the beneficial ones. Whether this effect is mediated through hypoglycemia’s impairment of collateral CBF or by other mechanisms remains to be determined. However, the results of our studies in cats should caution against the clinical use of insulin-induced hypoglycemia to prevent or ameliorate the brain damage of focal cerebral ischemia.

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References


Response: The question of the optimum levels of blood...
glucose levels for outcome after focal ischemia is a relevant one for patient care as well as for more basic science. We appreciate the thoughtful comments of Drs. de Courten-Myers, Wagner, and Myers and the opportunity to discuss in an open forum the possible reasons for the different results seen in their experiments and ours.

Often, differences in whole-animal experimental results can be traced to animal genetics (either to species or even to strain differences\(^1,5,10,14\)) or to methodology. The methodology used by de Courten-Myers and colleagues involved using metaanalysis of nine groups of their own animals,\(^6\) four of which (their Groups 2, 3, 8, and 9) were historical controls, having been published previously\(^3–5\) according to the legend to their Table 1. In their groups (1–6) with permanent ischemia, control Group 2 (receiving only saline before and after ischemia) precedes both Group 4 (10% glucose before ischemia, saline after ischemia, 5%) and Group 5 (10% glucose before and after ischemia). We used concurrently treated control groups, alternating experiments with the two test groups. The mortality differences in the five groups of de Courten-Myers, et al.,\(^6\) receiving permanent occlusion were one of 10, 0 of 13, two of 12, four of 13, and four of 10 in Groups 1 to 6, respectively. Although comparing mortality of four of 13 and four of 10 with 0 of 13 is indeed significant using Fisher’s exact test, this is true only if Groups 4 and 5 are all compared only to Group 2, with no Bonferroni correction factor applied. The Bonferroni correction factor for the number of possible intergroup comparisons would indeed be prohibitive, even if all groups are compared only to control Group 2 (probability values would have to be multiplied by five).

Based on these comparisons between groups, impressive increases in infarct size were reported with insulin-induced hypoglycemia by de Courten-Myers, et al.\(^6\) In their favor, all reported infarct sizes were carefully controlled for interanimal size variation by being expressed in percent rather than in cubed millimeters. Values were 55.9% in preischemically hyperglycemic animals whose glucose levels were later lowered with insulin versus only 5.2% in animals given saline before and after ischemia. Similarly, in transient focal ischemia (their Groups 7, 8, and 9) the differences found\(^6\) were also major: 57.8% in animals treated both preischemically and postischemically with insulin versus only 10.4% in animals given saline before and after ischemia and 12.2% in animals treated with 10% glucose before and after ischemia. However, once again there is a potential problem in using historical controls: Groups 8 and 9 preceded Group 7, having been published earlier, according to the authors’ legend to Table 1. Considering all nine groups in their paper\(^6\) three of the four historical groups (Groups 2, 8, and 9) show damage under 15% and are the only ones with damage in that range; the fourth historical group showed 48.9% damage (Group 3), having received 10% glucose both before/at and after MCA occlusion, a procedure that is expected to enhance brain damage. Although the authors offer a preemption of the above criticism of historical control in their Methods section, stating “The randomization of the groups was incomplete, but the reproducibility of the results has been demonstrated for two of the groups widely separated in time, with the mean of one group falling within one SEM of the other,” problems associated with the use of historical controls remain. Most investigators have experienced longitudinal inability to reproduce their results exactly over time. One factor in ischemic variability may be animal strain.\(^1,2,7,10,14\) Intraspecies differences are not limited to ischemia. Strain differences in fact can outweigh differences due to a genetic knockout,\(^15,16\) even in mice, which are genetically homogeneous compared to cats. Clearly, genetic background is an important experimental variable. Other differences with historical controls can possibly relate to seasonal changes in methodology, availability of animals, or operator. Some differences can be gradual over time, such as the differences in degree of interference with collateral circulation as a surgical experimenter increases in proficiency over time. Together, these factors may lead to intra- and interlaboratory variability through no fault of the experimenters, underscoring the need for concomitantly performed controls as closely genetically related as possible.\(^2\) Otherwise, reproducibility may be impossible. We wish to underscore the importance of randomized, concomitantly performed control studies.\(^2\)

Since publication of our article, one of us (R.N.A.) has conducted further experiments using concomitant controls to address the question raised regarding optimum levels of glycemia. Preliminary analysis (C. Zhu and RN Auer, unpublished observations) suggests that lowering blood sugar too much may indeed be detrimental, substantiating the concerns raised by Drs. de Courten-Myers, Wagner, and Myers. However, it also suggests, because we used rats, that a species difference (and differences in availability of collateral vessels between species) is not the explanation. Drs. de Courten-Myers, Wagner, and Myers also raise intriguing hypotheses in their letter. If hypoglycemia is indeed only protective in an “end-artery” or completely ischemic situation, then protection should be absent and damage could be enhanced by hypoglycemia, as has been observed,\(^13\) and reduced by hyperglycemia, as observed\(^11\) in the rim of selective neuronal necrosis around focal ischemia. However, this border zone is smaller in gyrencephalic animals and especially in humans,\(^2\) also countering the explanation presented by Drs. de Courten-Myers, Wagner, and Myers based on leptomeningeal collateral circulation being better in gyrencephalic animals. Hypoglycemia, if too severe, seems detrimental not only in larger-brained species. Another intriguing hypothesis raised in their discussion\(^6\) is that hypoglycemia stimulates the glycolytic capability of partially perfused tissue, leading to a paradoxically enhanced acidosis with hypoglycemia. This is another important hypothesis raised by Drs. de Courten-Myers, Wagner, and Myers, which needs testing.

We believe the curve relating blood glucose levels to brain damage is likely a U-shaped one, as implied by Drs. de Courten-Myers, Wagner, and Myers. Their concerns over generation of hypoglycemia in humans who have focal ischemia is certainly proper, and verbiage such as “may” and “possible relevance” is always used advisedly for this reason. It seems reasonably certain that high blood sugar levels should be decreased with insulin in focal ischemia, with only three dissenting papers in the literature indicating a benefit of high glucose levels,\(^8,9,17\) the remainder indicating detriment. However, the additional question of the level to which blood sugar should be...