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From Bedside to Bench and Back Again: Research Issues in Animal Models of Human Disease

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Abstract

To improve outcomes for patients with many serious clinical problems, multifactorial research approaches by nurse scientists, including the use of animal models, are necessary. Animal models serve as analogies for clinical problems seen in humans and must meet certain criteria, including validity and reliability, to be useful in moving research efforts forward. This article describes research considerations in the development of rodent models. As the standard of diabetes care evolves to emphasize intensive insulin therapy, rates of severe hypoglycemia are increasing among patients with type 1 and type 2 diabetes mellitus. A consequence of this change in clinical practice is an increase in rates of two hypoglycemia-related diabetes complications: hypoglycemia-associated autonomic failure (HAAF) and resulting hypoglycemia unawareness. Work on an animal model of HAAF is in an early developmental stage, with several labs reporting different approaches to model this complication of type 1 diabetes mellitus. This emerging model serves as an example illustrating how evaluation of validity and reliability is critically important at each stage of developing and testing animal models to support inquiry into human disease.

Keywords

translational research; animal models; hypoglycemia unawareness; diabetes complications

Many serious clinical problems investigated by nurse scientists require multifactorial research approaches, including the use of animal models, to improve patient outcomes. *Translational research* has been defined by the American Physiological Society as “the transfer of knowledge gained from basic research to new and improved methods of preventing, diagnosing, or treating disease, as well as the transfer of clinical insights into hypotheses that can be tested and validated in the basic research laboratory” (Hall, 2001, p. G1127). Although translational research has shown promise for understanding and treating human disease, impediments to fulfilling this potential include inadequate funding and a lack of available, skilled researchers (Hall, 2001; Pober, Neuhauser, & Pober, 2001). Nurse scientists with expertise in preclinical research are well positioned to help fill this gap through their clinical experience, knowledge of complex disease processes and patient responses, and holistic perspective.

Nurse researchers at “the bench” have informed clinical practice at “the bedside” through a variety of approaches, including animal research. Recently, Page (2004) observed the important contributions that nurse scientists can make to nursing knowledge provided they use “appropriate” animal models, but noted that discussions surrounding what is appropriate have

been incomplete. This article seeks to move this dialogue forward by describing research considerations for the development of valid, reliable animal models. For the purpose of this article, we will assume that the animal researcher has satisfied the ethical considerations inherent in the use of animals in research. These include proper orientation and instruction in the humane use and care of animals, strict adherence to protocols approved by their Institutional Animal Care and Use Committee, and observation of accepted guidelines and best practices as set forth in documents such as the National Research Council's *Guide for the Care and Use of Laboratory Animals* (1996) and Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (2004).

A major goal of ethically conducted animal research is to reduce the numbers of animals needed for a given project. Addressing issues of validity and reliability in animal models will enhance animal well-being by reducing the numbers of animals ultimately needed for significance. These concepts are presented in context of an example from our own research experience, a model that is still being developed by several labs in parallel, hypoglycemia-associated autonomic failure (HAAF). This model was chosen for illustrative purposes, as rates of hypoglycemia and HAAF are increasing in patients with diabetes, a disease that currently affects at least 15 million people in the United States. Additionally, as the model is in a relatively early phase of development, this overview and critique may be informative to nurse investigators considering model development to address a clinical problem.

Animal Models

Animal models bridge in vitro laboratory investigations and studies in humans (Dawkins & Stockley, 2001). An animal model is an organism with a genetic, naturally obtained, or induced pathological process that closely parallels the same condition in humans (Hau, Andersen, Rye-Nielsen, & Poulsen, 1989). Areas that must be addressed when deciding upon a model type include the choice of animal species and the influence of external factors such as caging, handling, and diet. A primary consideration is the anatomic similarity between the organ system (s) affected by the condition being modeled in the animal and that of humans, as anatomic differences are often accompanied by physiological and metabolic differences (Svendsen & Gottrup, 1998). In addition, many diseases seen in humans occur in animals as well, including diabetes. Often, spontaneous clinical cases in laboratory animals lead to inbreeding programs that produce animals with a given disease, such as rodents with autoimmune diabetes similar to human type 1 diabetes mellitus (DM), the BioBreeding rat, and nonobese diabetic mouse (Rabinovitch, 1994). External factors that are important when deciding upon a model include physical conditions such as temperature, humidity, noise and light intensity, biological pathogens, pain, and feed composition. Any or all of these factors can affect homeostasis or the functioning of the model during experimental manipulation. Unlike in the clinical setting, most of these aspects can be controlled within the laboratory, an advantage of animal models when studying complex disorders.

Animal models are designed to be as analogous as possible to the clinical problem, and useful [animal] models have sets of causally related factors (Overmier, 1999). This causal chain begins with an initial analogy/initial hypothesis, and from that initial analogy additional hypotheses build to form formal analogies. Using hypoglycemia as an example, the initial analogy in the modeling process is that rodent and human responses to hypoglycemia are similar in important ways.

Treit (1985) described useful models as being of three general types: correlational, isomorphic, or homologous. Correlational animal models must meet three criteria: (a) the animal must respond to a pharmacologic agent known to treat the condition in a dose-dependent manner, (b) the relative potency of the drug should be comparable in the animal to that seen clinically,

and (c) the animal model should be selective (e.g., a depression model should be responsive to antidepressants). Correlational models often have problems of false negatives. For example, drugs that inhibit pentylenetetrazol-induced seizure activity in rodents will also frequently serve as predictors of drugs that will be an anxiolytic in humans (Swinyard & Castellion, 1966). As such, this model has become a method of selecting drugs for further testing. This screening process, however, would miss nonantiepileptic compounds that could ultimately prove successful.

Isomorphic models show overt similarities between human and animal pathology. In some models, such as induction of hypoglycemia by injection of insulin, this is straightforward, as the animal readily demonstrates hypoglycemia. On the other hand, in rodent models of mental health-related conditions such as anxiety and depression, behaviors relating to the human conditions are often much more difficult to assess, thus behavioral isomorphism may not exist or may not be readily quantifiable.

In homologous models, the researcher tries to specify the underlying cause of a test response based upon what is seen clinically in humans and must make assumptions of parallel causes and effects between animals and humans to make this model valid. Thus, the most successful animal models have generally been homologous and have taken advantage of similarities between the human disease or condition and the animal model to improve patient outcomes (Page, 2004).

Establishing Validity of Animal Models

In establishing the validity of animal models, three types of validity are examined: face validity, predictive validity, and construct validity.

Face Validity

In assessing the face validity of the animal model, the researcher examines the similarity in symptoms between the model and the condition being modeled (Jenck, Moreau, & Martin, 1995; Willner, 1990). The model and disorder should have similar diagnostic characteristics, with congruence of pertinent physiologic measurements (Feldman, Meyer, & Quenzer, 1997; Willner, 1990). In essence, the question to be considered is this: Does the model model what it is supposed to be modeling? Evaluation of face validity of an animal model involves measuring as many outcome variables as is feasible and conducting a pair-wise comparison with homologous outcome variables from human studies (see below).

The degree of descriptive similarity between two physiologic or behavioral states determines the degree of material or conceptual equivalence and is an important component of face validity (Overmier, 1999). For example, the principal adrenal glucocorticoid in the rat is corticosterone, whereas in humans it is cortisol. These two hormones are not chemically identical, though they are related, but they do have a high degree of material equivalence beginning with the fact that each is the final signal in the hypothalamo-pituitary-adrenal (HPA) axis. There are differences in receptor affinity between species such that species that secrete primarily cortisol have glucocorticoid receptors that bind cortisol with higher affinity than corticosterone. On the other hand, species such as mouse and rat that secrete predominantly corticosterone have glucocorticoid receptors that bind corticosterone with higher affinity than cortisol (Giannopoulos & Keichline, 1981; Sutanto & De Kloet, 1987).

With respect to stress responses, it is important to note that, in both humans and rats, the brain contains two types of receptors that bind glucocorticoids (Reul & De Kloet, 1985; Yu, Romero, Gomez-Sanchez, & Gomez-Sanchez, 2002). Mineralocorticoid receptors (MRs) have approximately equal affinity for aldosterone, cortisol, and corticosterone. In the brain, MRs

are mainly restricted to the hippocampus where they bind with high affinity to glucocorticoid hormones. On the other hand, brain glucocorticoid receptors (GRs) have a wider anatomical distribution and have much lower affinity for glucocorticoid hormones. GR occupancy is low unless high stress-induced levels of glucocorticoid hormones are reached (Reul & De Kloet, 1985). Both receptor types function via typical steroid hormone mechanisms, including binding to a nuclear receptor and altering cell transcription and translation in addition to possibly having rapid-acting, nongenomic activity (De Kloet, Vreugdenhil, Oitzl, & Joëls, 1998).

Conceptual equivalence occurs in the notion of exercise between rodents and humans if a rat has to climb on a wheel and rotate and a human has to use a treadmill; both require prior thought and physical effort to attain the goal of aerobic exercise. Although both material and conceptual equivalence offer the promise of a model's fidelity, they ensure nothing and are not always critical to the functional validity of a model (Overmier, 1999). Therefore, it is essential to assess the extent of an animal model's validity by additional methods.

Predictive Validity

Predictive validity determines the success of predictions made from the model and has generally referred to pharmacologic responses to drugs (Jenck et al., 1995; Willner, 1997). However, predictive validity of an animal model encompasses other responses such as short- and long-term consequences of the condition or disease. In terms of the usefulness of an animal model to clinical care of humans, predictive validity often aims to answer the question, Does the model correctly predict interventions that will be successful in the clinical setting? Predictive validity can be difficult to satisfy in pharmacologic research, as often therapies that show great promise in vitro or in animal models in vivo do not produce the desired effects in clinical trials (Littman & Williams, 2005; Thompson et al., 2005).

Construct Validity

Construct validity ensures that there is a convincing theoretical rationale for the model and assesses the degree to which the characteristics of the animal model can unambiguously be interpreted as being similar to those of the clinical condition (Jenck et al., 1995; Willner, 1997). Additionally, it is important to determine if the model's attribute maintains an established empirical and theoretical relationship to the clinical problem being modeled (Willner, 1984). Basically, the question to be answered here is, Do the internal workings of the model behave in a manner similar enough to the clinical situation being modeled that it will respond to experimental manipulations in the same manner as that clinical situation?

Productive Generativity

Animal model validity notwithstanding, it is possible to gain new insight into clinical problems even without demonstrating full construct, face, or predictive validity of the model. Shapiro (1998) stated that the power of a model is not in its validity but in its "productive generativity" (p. 89). The true critical point of a model is whether or not it generates new hypotheses or new understanding about the original clinical problem. Fundamentally, has the model led to further understanding of the original clinical problem? If so, the model is powerful and useful.

Establishing Reliability of Animal Models

The reliability of an animal model is established by demonstrating that under defined testing conditions, results are the same from one time to another within the same laboratory and from one laboratory to another (Feldman et al., 1997; Willner, 1997). This criterion can be quite difficult to achieve as many factors may influence the reliability of results even within the same model. Differences in strains of animal, vendors, lighting, diet, gender, age, parity, and animal

handling and care practices can result in variations in results (Willner, 1997). It may also be difficult to determine congruence in animal models between various laboratories based on the limited details usually provided within published manuscripts regarding animal handling and care or laboratory use of standard protocols.

Using the above evaluation criteria for validity and reliability of animal models, the HAAF model will first be described and then analyzed.

Hypoglycemia-Associated Autonomic Failure: Progress in Model Development

The physiological response to hypoglycemia depends in great degree on the central nervous system. The state of hypoglycemia produces activation of the sympathetic nervous system, which promotes two potent endocrine responses: increased adrenomedullary epinephrine secretion and augmentation of pancreatic glucagon secretion. Additional effects of sympathetic nervous system activation during hypoglycemia include tachycardia and diaphoresis. The parasympathetic nervous system is also stimulated by hypoglycemia, specifically increasing vagal outflow to the stomach to increase gastric motility. These autonomic and endocrine responses give rise to many of the classical signs and symptoms of hypoglycemia such as palpitations, nervousness, sweating, and hunger. In addition to these autonomically mediated responses, two additional endocrine systems respond to hypoglycemia with increased secretion, namely, the HPA axis and pituitary growth hormone. Thus, the counter regulatory response facilitating recovery from hypoglycemia involves the hormones glucagon, epinephrine, cortisol, and growth hormone. Symptom perception by the central nervous system also engenders a behavioral response (food seeking, eating) that contributes to recovery from hypoglycemia (Cryer, 1997).

Effective management of DM involves insulin administration to reduce hyperglycemia and restore euglycemia; however, lack of precision in insulin replacement often leads to recurrent hypoglycemic episodes, particularly in patients with type 1 DM. For this reason, hypoglycemia has been described as the “limiting factor” in effective management of DM (Cryer, 1994). Although generally associated with insulin treatment of type 1 DM, hypoglycemia is also a complication of type 2 DM in cases that are intensively managed with insulin or insulin secretagogues such as sulfonylureas. Intensive management of diabetes involves the use of an insulin pump or multiple daily insulin injections, as well as frequent self-blood glucose monitoring, with the goal of maintaining blood glucose as close to normal as possible. Randomized clinical trials of intensive management of type 1 DM (the Diabetes Control and Complications Trial) and type 2 DM (the United Kingdom Prospective Diabetes Study) demonstrated significant reductions in long-term diabetic (primarily microvascular) complications in patients using intensive management. However, in both of these clinical trials, severe hypoglycemia was more frequent in the intensive-management groups (Diabetes Control and Complications Trial Research Group, 1993, 1997; United Kingdom Prospective Diabetes Study Group, 1998). Recurrent episodes of hypoglycemia often result in hypoglycemia unawareness, the inability to consciously perceive the classical warning signs of low blood glucose (Gerich, Mokan, Veneman, Korytkowski, & Mitrakou, 1991). Patients with intensively managed DM are at high risk for hypoglycemia unawareness, causing them to be vulnerable to hypoglycemic coma and seizures.

The physiological basis of hypoglycemia unawareness appears to involve a progressive reduction in autonomic and endocrine responses to hypoglycemia, with a loss of appropriate secretion of the counterregulatory hormones glucagon, epinephrine, cortisol, and growth hormone. Of these hormones, levels of glucagon and epinephrine are of primary importance in rapid restoration of blood glucose in nondiabetic individuals (Cryer, 1997). However, due

to alterations within the islets of Langerhans, patients with type 1 DM lack a glucagon secretory response to hypoglycemia and are thus dependent on epinephrine secretion to combat hypoglycemia (Cryer & Gerich, 1983). Recurrent hypoglycemia is associated with progressive blunting of autonomic responses to hypoglycemia, particularly manifested as a deficient epinephrine secretory response to hypoglycemia, giving rise to the phenomenon of HAAF (Cryer, 2001). Loss of the autonomic and endocrine responses to hypoglycemia also contributes to the lack of symptoms, causing hypoglycemia unawareness. As intensive management becomes widely accepted as the standard of diabetes care, fear of hypoglycemia represents a major barrier to client adherence (Ramchandani et al., 2000; Reach, Zerrouki, Leclercq, & d'Ivernois, 2005).

Recent and/or recurrent episodes of hypoglycemia are strongly implicated in the pathogenesis of both HAAF and hypoglycemia unawareness (Cryer, 2004). The neural mechanisms linking recurrent hypoglycemia to HAAF and hypoglycemia unawareness are not understood at this time, and the only effective intervention is to relax intensive management for several weeks in order to reduce recurrent hypoglycemia (Dagogo-Jack, Fanelli, & Cryer, 1999; Fanelli et al., 1993). These clinical problems are associated with biological, psychological, and social attributes and are best addressed by a number of research approaches including clinical research and basic science.

The biological attributes of HAAF have material validity to study in a rodent model, as hypoglycemia in rats stimulates secretion of epinephrine, glucagon, and corticosterone, all of which can be sampled in rat blood and measured by standard laboratory assays. Although these responses appear to be isomorphic with human responses, issues of validity and reliability of rodent hypoglycemia studies are important and have not been addressed in the research literature to date.

A major limitation in attempting to model HAAF in rodents relates to reproducing technical aspects of the human studies in such a small animal. Specifically, the method used to evaluate human responses to hypoglycemia involves the use of the hyperinsulinemic stepped hypoglycemic clamp. In these experiments, human patients are instrumented with an indwelling venous catheter for drug infusion in one arm and a blood-sampling catheter inserted in the retrograde direction in a forearm vein of the other arm. The arm used for blood sampling is placed in a heated box that favors blood flow through arteriovenous shunts; therefore, the samples collected are considered to be "arterialized" venous blood. Thus, if hypoglycemia alters capillary glucose removal rates, the blood levels of glucose from the sampling arm should not be altered. At a constant rate of insulin infusion, glucose is infused simultaneously to maintain baseline blood glucose concentration. Then, the glucose infusion rate is progressively reduced, with frequent blood glucose measurements, allowing blood glucose to fall in a stepwise fashion to hypoglycemic levels. For example, blood glucose will be maintained at 75 mg/dl for 1 hr followed by 65 mg/dl, 55 mg/dl, and 45 mg/dl. Time is allowed at each level for hormone levels to stabilize; then blood samples are drawn for measurements of glucagon, epinephrine, norepinephrine, cortisol, growth hormone, and pancreatic polypeptide (an indicator of parasympathetic stimulation of the pancreas). With the use of this elegant technique, it has been shown that prior hypoglycemia in humans results in (a) decreased peak hormone levels at each blood glucose step and (b) shift of the blood glucose threshold for initial counter-regulatory hormone release to progressively lower levels (Dagogo-Jack, Craft, & Cryer, 1993; Widom & Simonson, 1992).

Although the use of the hypoglycemic clamp is ideal in human studies for measuring both hormone responses to hypoglycemia and glucose thresholds for hypoglycemia-induced responses, there are significant feasibility issues when attempting to use this technique in conscious, behaving rats. Due to technical limitations of vascular access and catheter patency,

studies in rats may use a single catheter for sampling or two catheters for clamping (jugular venous catheter for infusions and a carotid artery catheter for sampling). Hypoglycemic clamp measurements in rats also generally use a single level of hypoglycemia rather than using the stepped-clamp approach. Given the much smaller blood volume of a rat, there are limitations as to the volume that can feasibly be sampled for hormone measurements without inducing hypovolemic stress in addition to hypoglycemia.

The rats are surgically prepared under anesthesia using sterile surgical technique to implant the vascular catheters. The experiments take place after several days of postoperative recovery, with rats connected to tubing to allow remote blood sampling in a manner that attempts to reduce stress. As epinephrine and corticosterone are both stress hormones, it is essential that the rats be maintained in the best of health and habituated to the experimental conditions before the measurements of hormone responses to hypoglycemia are conducted.

To provide greater methodological detail, in the work of Tkacs and colleagues, the catheter is exteriorized between the scapulae and is plugged with a short metal occluder. The orientation of the catheter is such that the rats are unable to reach it and chew on it while grooming. It should be noted that this instrumentation requires that rats be individually housed, as cagemates would chew one another's catheters. During habituation and hormone-sampling experiments, the occluder is removed and the catheter is attached to saline-filled extension tubing that leads outside of the cage through a perforated lid. Rats are connected thus for at least 2 hr daily for 2 to 3 training days before the hypoglycemic challenge in order to habituate them to the recording conditions. When handled in this manner, baseline plasma epinephrine measurements are below detectable levels, indicating that acute stress is likely to be minimal (Tkacs, Dunn-Meynell, & Levin, 2000; Tkacs, Pan, Raghupathi, Dunn-Meynell, & Levin, 2005). Readers are also referred to a recent authoritative review on this subject that concluded that blood sampling from indwelling vascular catheters in rats produces less stress than tail-vein sampling (Vahl et al., 2005).

Face Validity of Rodent HAAF Studies

Research studies in human adults without diabetes have established that autonomic, endocrine, and symptom responses to hypoglycemia decrease after prior hypoglycemia. This reduction in counterregulatory responses occurs after as little as one episode of hypoglycemia (Heller & Cryer, 1991; Robinson, Parkin, MacDonald, & Tattersall, 1995). Other investigators demonstrated this phenomenon after repeated episodes of hypoglycemia delivered on consecutive days (2 days, blood glucose averaging 45 mg/dl for 60 min each day) (Widom & Simonson, 1992) or two episodes in a single day involving morning and afternoon sessions (hypoglycemia ranging from 52 to 70 mg/dl maintained for up to 2 hr in each session) (Davis et al., 2000; Davis, Shavers, Mosqueda-Garcia, & Costa, 1997). These studies support the concept that recent hypoglycemia acutely induces HAAF in humans (Cryer, 2001). Depth of the initial hypoglycemic episode, rather than duration, appears important in down-regulation of subsequent responses—the lower the blood glucose during the initial episode, the greater the suppression of epinephrine secretion during a subsequent hypoglycemic challenge (Davis, Shavers, Mosqueda-Garcia, & Costa 1997; Davis et al., 2000). All of these studies report suppression of hypoglycemia-induced epinephrine secretion after prior hypoglycemia, and most report similar declines in hypoglycemia-induced norepinephrine, glucagon, growth hormone, and cortisol secretion. When hypoglycemic symptoms were assessed, these responses were also reduced after prior hypoglycemia (Davis et al., 2000; Heller & Cryer, 1991; Widom & Simonson, 1992).

In rats, growth hormone does not appear to contribute to the counterregulatory response; however, hypoglycemia induces pronounced increases in epinephrine, glucagon, and corticosterone secretion. Just as the human studies described above have used differing depths,

durations, and repeated presentations of hypoglycemia, researchers using rodent models have used a variety of approaches to evaluate the effect of prior hypoglycemia on hormone responses to subsequent hypoglycemic challenge. Hypoglycemia-induced epinephrine secretion was noted to be markedly reduced 2 days after an initial episode of hypoglycemia (36 mg/dl, duration < 1 hr) elicited by insulin 5 U/kg IV (Tkacs et al., 2000). In the same study, hypoglycemia-induced corticosterone secretion was modestly but significantly blunted, whereas norepinephrine levels were unchanged, and glucagon was not measured. The finding of blunted hypoglycemia-induced epinephrine secretion in rats subjected to prior hypoglycemia has been confirmed by several other research groups. In one such study, rats were given 1.5 U insulin subcutaneously on 2 consecutive days to reduce blood glucose to 26 mg/dl (average on day 1) and 32 mg/dl (average on day 2) (Sivitz et al., 2001). However, in this study, blood glucose was only measured 150 min after insulin injection; thus, the depth and duration of hypoglycemia was not closely monitored and corticosterone and glucagon were not measured. Another research group used hypoglycemic clamp in rats to evaluate the effect of 3 days of recurrent hypoglycemia induced by intraperitoneal insulin injection, with hypoglycemia (down to 40 mg/dl) lasting up to 150 min each day. This treatment was associated with significantly reduced epinephrine and norepinephrine elevations to hypoglycemic clamp, whereas glucagon and corticosterone were not significantly suppressed (Flanagan et al., 2003). In general, this phenomenon has been reproduced in several research laboratories studying rat responses to recurrent hypoglycemia (de Vries, Lawson, & Beverly, 2004; Evans et al., 2001; Shum et al., 2001; Tkacs et al., 2005).

In humans and rats alike, the most robust marker of a blunted autonomic and neuroendocrine response to hypoglycemia is diminished epinephrine secretion, whereas glucagon and corticosterone responses appear more variable. In both humans (Heller & Cryer, 1991) and rats (Tkacs et al., 2000, 2005), as few as one episode of hypoglycemia has been shown to reduce epinephrine secretion to subsequent hypoglycemia. Thus, there is substantial face validity in that experimental rat studies of recurrent hypoglycemia produce results similar to experimental human studies. One obvious limitation of rodent studies is the inability to assess hypoglycemic symptoms, an important component of the human studies. Thus, the interpretation of rat studies is limited to the quantifiable effects of recurrent hypoglycemia on indicators of endocrine and autonomic function. Although this is admittedly a limitation, the deficit in epinephrine secretory responses observed in rats exposed to recurrent hypoglycemia is homologous to the human studies and as such is a strong argument for face validity of the model.

To reduce variability, the human and rodent studies summarized above were carried out in individuals without diabetes; however, the effect of prior hypoglycemia reducing responses to subsequent hypoglycemia has also been demonstrated in patients with diabetes (Dagogo-Jack et al., 1993; Ovalle et al., 1998; Rattarasarn, Dagogo-Jack, Zachwieja, & Cryer, 1994). Studies in diabetic rats have also confirmed that recurrent hypoglycemia reduces endocrine, and particularly epinephrine, secretory responses to hypoglycemia (Inouye et al., 2002; Powell, Sherwin, & Shulman, 1993). Based on the preponderance of the evidence, it now appears reasonable to assume that blunted neural, endocrine, and symptom responses to hypoglycemia associated with intensive management of type 1 DM are due, at least in part, to recurrent hypoglycemia in this population (Amiel, Tamborlane, Simonson, & Sherwin, 1987).

The substantial advantage of the rodent model is the ability to follow up the hormone sampling phase of an experiment with euthanasia and postmortem examination of the brain and other relevant tissues to study correlated neuropathology and associated alterations (Tkacs et al., 2000, 2005; Yamada et al., 2004). An additional advantage is the ability to induce consistent and significantly low levels of hypoglycemia. Ethical and safety concerns dictate that HAAF research studies in human volunteers evaluate responses to blood glucose levels in the range of 45 to 50 mg/dl. However, studies of adults and children with diabetes have documented

blood glucose levels as low as 30 mg/dl during spontaneous hypoglycemia, particularly in episodes occurring at night that do not result in awakening (Matyka, Wigg, Pramming, Stores, & Dunger, 1999). Furthermore, there is a significant incidence of seizure and coma in intensively managed adolescents and adults with DM, with presumed blood glucose levels of 15 to 20 mg/dl. Some of the rodent HAAF studies have used blood levels as low as 30 mg/dl, a level close to that often experienced by individuals with type 1 DM.

Similarly, children with diabetes frequently experience spontaneous hypoglycemia, particularly nocturnal hypoglycemia, but it is not ethical to conduct studies of induced hypoglycemia in children with, or without, diabetes. Thus, recent hypoglycemia studies in juvenile rats take on added significance (Yamada et al., 2004; Yamada, Rensing, & Thio, 2005). Here there is a philosophical quandary—the face validity of the rodent HAAF model is established, to a certain degree, by comparison with the above-mentioned human studies, but rat experiments can be extended in ways that may be clinically relevant but not directly comparable to human studies. This is an example of the potential for productive generativity of an animal model in which there may not be an opportunity to fully demonstrate validity in correlation with human studies.

Predictive Validity of Rodent HAAF Studies

As use of rats as a model of HAAF is in its relative infancy, there is little information with respect to the predictive validity of this model and there are many questions that remain to be answered. Intervention studies in patients with hypoglycemia unawareness indicate that hypoglycemic symptoms return when hypoglycemia is carefully avoided for several weeks. Furthermore, neural and endocrine responses to hypoglycemia improve when hypoglycemia is avoided, but complete restoration to the levels of individuals without diabetes is not achieved, indicating that some of the adaptation to recurrent hypoglycemia involves an irreversible process (Dagogo-Jack et al., 1999; Dagogo-Jack, Rattarasarn, & Cryer, 1994; Fanelli et al., 1993). Of the rat studies cited above, only Powell and colleagues (1993) evaluated recovery of hypoglycemia-induced epinephrine secretion over time after severe recurrent hypoglycemia (daily episodes for 1 month). Powell and colleagues reported a partial return of the epinephrine secretory response to hypoglycemia 4 weeks after cessation of recurrent hypoglycemia. This observation is consistent with the human literature, thus supporting predictive validity of the rodent model for human HAAF. With respect to intervention studies, a recent report provided histological evidence of neuroprotection by ketogenic diet in a juvenile rodent moderate hypoglycemia model (Yamada et al., 2005); however, preservation of hormone responses to hypoglycemia after the intervention was not assessed. To date, there have been no reports of pharmacological studies of neuroprotective agents that prevent HAAF or reduce its severity in humans or rodents.

Construct Validity of Rodent HAAF Models

Studies of a mechanistic nature that can readily be carried out in animal models provide a necessary counterpoint to human studies of clinical phenomena, wherein each approach can stimulate complementary studies related to construct validity. With respect to theoretical aspects of HAAF research, there is substantial evidence for human and animal studies being mutually informative. With this in mind, two theoretical aspects of HAAF research will be considered. One hypothesis attributes loss of responses to recurrent hypoglycemia to the effect of elevated cortisol levels attained during hypoglycemia. Tests of this hypothesis have been carried out in humans and in rats, with conflicting results. In humans, Davis, Shavers, Costa, and Mosqueda-Garcia (1996) noted that hypoglycemia-induced epinephrine secretion was reduced after prior cortisol treatment (two 2-hr infusions). Furthermore, prior hypoglycemia did not reduce epinephrine secretion during subsequent hypoglycemia in patients with adrenocortical failure in whom there was no cortisol response to prior hypoglycemia (Davis,

Shavers, Davis, & Costa, 1997). These results have not been fully replicated. Another lab demonstrated cortisol-induced suppression of responses to hypoglycemia in human patients treated with high doses of adrenocorticotrophic hormone to produce pharmacological cortisol levels (McGregor, Banarer, & Cryer, 2002). However, treatment with elevated levels of cortisol in the range normally measured during hypoglycemia did not reduce hypoglycemia-induced epinephrine secretion in a follow-up study (Raju, McGregor, & Cryer, 2003). Failure of glucocorticoid pretreatment to suppress responses to subsequent hypoglycemia has been reported in rodent studies. The primary endogenous glucocorticoid in rats is corticosterone rather than cortisol. Similar to the results of Raju and colleagues in humans, elevation of corticosterone in rats did not reduce epinephrine secretion levels in response to subsequent hypoglycemia (Evans et al., 2001; Flanagan et al., 2003; Shum et al., 2001). On the other hand, administration of cortisol into the lateral ventricle in rats was reported to reduce hypoglycemia-induced epinephrine secretion (Sandoval, Ping, Neill, Morrey, & Davis, 2003). Thus, human and rodent studies investigating the cortisol hypothesis as a possible mechanistic basis of HAAF are in progress and are being refined in an attempt to more closely match the clinical situation.

Other human studies have demonstrated that hormone, symptom, and cognitive responses to hypoglycemia are reduced when lactate is infused intravenously concurrently with induction of hypoglycemia (Maran, Cranston, Lomas, Macdonald, & Amiel, 1994; Maran et al., 2000; Veneman, Mitrakou, Mookan, Cryer, & Gerich, 1994). The hypothesis is that the lactate represents an alternative fuel for brain energy supplies. A similar result has been reported in rats in a study demonstrating that lactate infusion into the brain via the cerebral ventricles reduces brain Fos staining (a marker of neuronal activation) in response to systemic 2-deoxy-D-glucose (2-DG) (Briski, 1999). 2-DG is a molecule that induces intracellular glucose deprivation, mimicking hypoglycemia. These studies with glucocorticoid and lactate infusions in rats demonstrate the utility of a rodent model in testing hypotheses of the pathogenesis of HAAF and provide beginning evidence of construct validity of the rodent model.

Reliability of Rodent HAAF Studies

The reliability of the rodent HAAF model appears well established. The epinephrine secretory response to hypoglycemia can be reduced by either single (Tkacs et al., 2000, 2005) or repeated episodes of insulin-induced hypoglycemia. Single episodes in which blood glucose is maintained between 30 and 35 mg/dl for at least 1 hr appear to be sufficient to produce the phenomenon, whereas a single episode at approximately 50 mg/dl reportedly does not reduce epinephrine secretion to subsequent hypoglycemia (Paranjape & Briski, 2004). Inasmuch as both depth and duration of hypoglycemia appear to be critical parameters in these experiments, it is important to measure blood glucose frequently (every 20–30 min) and with an instrument that is validated for low levels of blood glucose. It is expected that rodent HAAF studies will now progress to a focus on intervention development and testing, with the aim of reducing this serious and distressing complication of diabetes treatment.

Conclusion

It is important that investigators using animal models have an understanding of the concepts that underlie model development. These include ethical considerations, appropriate species choice, and the rigorous regulation of environmental factors. Establishment of validity and reliability of models used in translational research is an essential aspect of research design. Face validity of a model is established if measurements made in the model faithfully correlate with the identical or homologous variables assessed in the disease condition. Predictive validity, which is necessary for pharmacologic or interventional testing, can be the most difficult to achieve and has not yet been demonstrated for HAAF. Construct validity examines

the theoretical rationale of the model for congruence to the clinical problem; both human studies and rodent HAAF models are being pursued in parallel to test several hypothesized mechanisms of HAAF (as reviewed in Cryer, 2005). Reliability of models is also important to establish but may be difficult to assess between laboratories if the model is relatively new, as with the HAAF models, or if any experimental practices vary widely.

Careful determination of the validity and reliability of the animal model serves several functions: It provides a strong conceptual framework for the ongoing research, it strengthens the rationale for the ethical use of animal models in nursing research, and it provides strong evidence that research done at the bench can impact care at the bedside.

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