Diabetologia 6, 366–370 (1970)

Further Studies on the Effect of Hypothalamic Lesions in the Sand Rat (Psammomys Obesus)

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Summary. Median eminence lesions, in the area of the arcuate nucleus, appear to decrease, one hour post oral glucose, blood glucose levels in hyperglycemic rats, independent of changes in weight.

Nouvelles observations quant à l'effet de lésions hypothalamiques chez le rat des sables (Psammomys obesus)

Résumé. Des lésions placées au niveau du nucléus arcuatus semblent diminuer les taux de glucose sanguin observés une heure après une surcharge par voie orale chez les animaux par ailleurs hyperglycémiques.

Zusätzliche Beobachtungen der Folgen hypothalamischer Laesionen bei der Sandratte (Psammomys obesus)

Zusammenfassung. Laesionen in der Gegend des Nucleus Arcuatus scheinen die nach einer Stunde nach Glucose *per os* erhaltenen Blutzuckerwerte bei hyperglykämischen Sandratten zu vermindern.

Key-words: Sandratte, Psammomys obesus, blood glucose, hypothalamus, lesions of hypothalamus, hyperglycemia.

In an attempt to establish a laboratory colony of the sand rat (Psammomys obesus), it was found that this animal develops a chow-induced diabetic syndrome [29]. This syndrome is characterized by hyperglycemia, elevated plasma insulin levels except during ketoacidosis, diminished extractable pancreatic insulin, glucosuria, and pathologic lesions including cataracts, B cell degeneration, vacuolization of the pancreatic islet tissue and glycogen nephrosis [13, 14, 25, 20, 11]. The animals vary in their diabetogenic susceptibility [14, 24].

That starvation diminishes carbohydrate tolerance was first recognized by Claude Bernard [3], and has been since confirmed by a number of investigators [32, 2, 17, 35]. The possible existence of a long acting contra-insulin starvation hormone from the anterior pituitary was considered over thirty years ago [10, 2]. In white rats, 7—10 days are required for the development of starvation diabetes, these animals being notably resistant to the induction of experimental diabetes [17]. The effect of a preliminary feeding of carbohydrate in improving the glucose tolerance was first described in 1919 [15] and by Staub and Traugott [31, 34]. This effect has also been observed in the sand rat, with the blood sugar returning toward starvation levels within 6 h in this species (see below).

It has been known since the fundamental observation of B.A. Houssay and his collaborators [16], that removal of the anterior lobe of the pituitary greatly diminishes the severity of diabetes in pancreatectomized animals. F. G. Young, in his studies on metahypophyseal diabetes, established the concept that growth hormone (GH) might be the diabetes-inducing substance of the hypophysis [8].

In 1963 [28], it was shown that insulin-induced hypoglycemia was associated with a rapid rise in plasma GH levels which could be shut off by prompt glucose administration. Also, prolonged fasting in a normal subject was accompanied by a progressive rise in the plasma level of the hormone, subsequently turned off by feeding. The fasted sand rat with chemical diabetes has relatively low fasting and 4 h blood glucose values. It seemed possible, therefore, that GH was playing a role in the development of diabetes in the sand rat, since fasting and feeding in these intermittent feeders could provide continuous alternations of blood sugar levels as a stimulus to GH secretion.

Hypothalamic releasing factors for GH have been demonstrated [27, 18, 21], as have hypothalamic glucoreceptors [23, 22, 1]. The possibility that an increased sensitivity of these receptors to a blood sugar fall, or to some other metabolic stimulus appearing in the course of starvation played a role in initiating the diabetic syndrome in the sand rat was considered by us; if this were so, interruption of the hypothalamo-hypophyseal reflex arc might have an ameliorative effect. Reflex secretion of GH in the rat appears to be blocked by lesions in the posterior median eminence [19]. Our earlier studies have suggested that this type of lesion may improve the diabetic glucose tolerance of the sand rat [4, 5].

This paper describes some characteristics of the oral glucose tolerance of 94 sand rats, and a correlative study of post-lesion histology, 1 h blood glucose levels after oral glucose, and weight, in 17 sand rats.

Materials and Methods

Sand rats trapped in Egypt, and others bred in captivity, were used. All animals received fresh vegetables (carrots and spinach) ad libitum, with no additional water available. They were kept in a separate room in the animal quarters kept at 78—82° F and 40—50% relative humidity. Glucose 300 mg/ml N saline/100 g rat was administered orally by gavage after an overnight (15—17 h) fast, and glucose levels

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were measured in 20 μl of blood obtained from the tip of the tail. "Two and six hour fed" sand rats were fasted overnight, then given oral glucose two and six hours after feeding of vegetables, ground up with N saline, in a Waring blendor. "Two hour fed" white rats were treated in a similar manner but were fed a suspension of lab chow in saline also prepared in a Waring blendor. Blood sugars were measured on the Technicon autoanalyzer using the standard ferricyanide microdetermination technique.

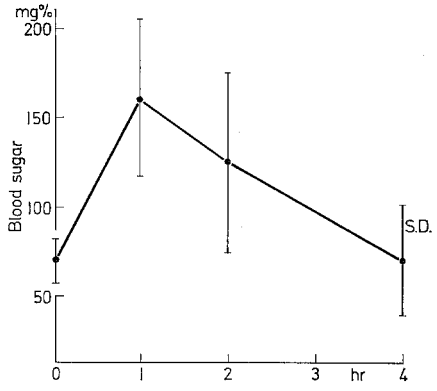

Fig. 1. Oral GTT in 94 sand rats

A radiofrequency lesion maker "Grass model LM3" was used for placing the stereotaxic lesions. It had a stainless steel electrode diameter of 0.3 mm, insulated with epoxylite, except for 0.4 mm at the tip. The animal's head was fixed in a C.A. Stoelting stereotaxic instrument and the incisor bar adjusted so that the surface of the animal's skull was perpendicular to the vertical zero of the instrument. Animals were anaesthetized with Avertin (25 mg/100 gm rat IP). The stereotaxic coordinates for the lesions were F=4.8 (except if noted otherwise) H=base+0.2, placed in the midline. A current of 3 milliamperes (estimated) was passed for 30 sec. Animals were either decapitated terminally or, in some cases, died from other causes. Brains were fixed in 10% formalin and prepared for histological examination either by passage through alcohol and embedding in paraffin, or by freezing. Sections (15 μ thick for paraffin and 50 μ for frozen) were stained for the most part with toluidine blue but H&E, thionine and cresyl violet were also used. Histology was read blind (G.Z.).

Results and Discussion

The four hour oral glucose tolerance of 94 sand rats shows a peak at 1 h, relatively high when compared to white rats, and low fasting and 4 h values. (Fig. 1). The distribution of one hour blood sugars appears to be unimodal but skewed, with many animals in the hyperglycemic range (Fig. 2). There is no correlation between weight and one hour blood sugars (Fig. 3). Information on body composition in the sand rat is

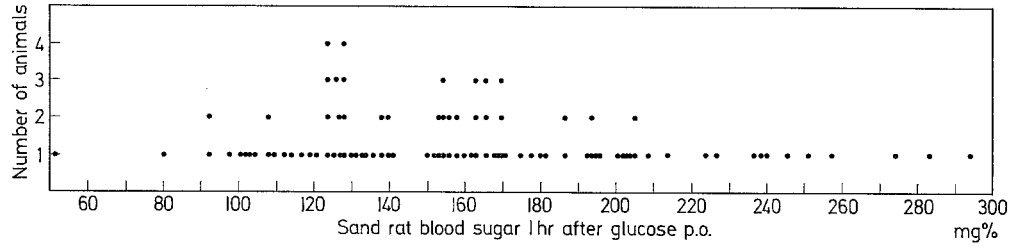

Fig. 2. Distribution of 1 h blood sugars in the sand rat (94 animals)

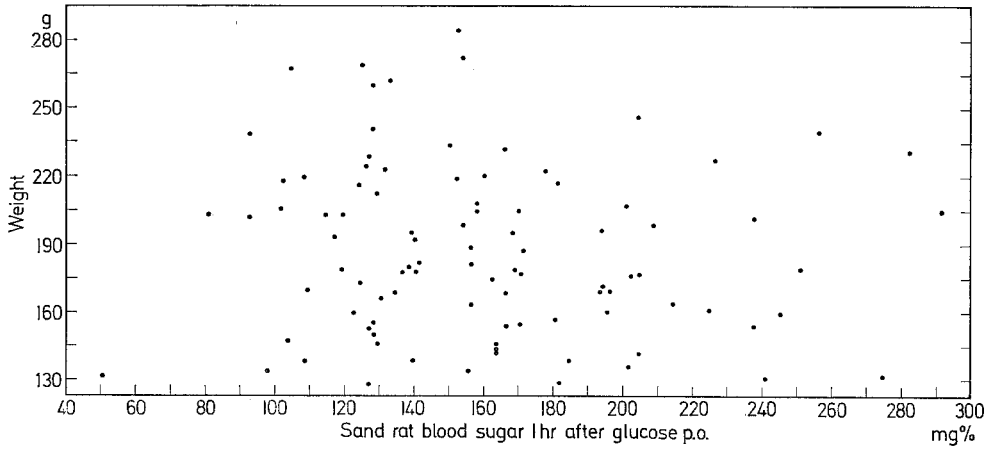

Fig. 3. Relationship to weight of 1 h blood sugar levels in the sand rat (94 animals)

unavailable at present. Prior feeding lowers the 1 h blood sugar somewhat in hyperglycemic sand rats, but not in white rats or normoglycemic sand rats (Table 1). Furthermore, this is throughly dissipated (within 6 h) even in the hyperglycemic sand rat (Table 1). Although the mechanism of the Staub Traugott effect remains to be elucidated [33, 9], the hypophysis appears to be indispensable for this phenomenon [30].

ciated with a transitory drop in blood sugar (lasting 64 days) and the other animal demonstrated an electrode tract in the median eminence with questionable destruction of cells. Midline lesions in other areas of the anterior and posterior hypothalamus did not alter the blood sugar in 8 animals (Table 3).

There are a number of problems in the interpretation of these data. Since this study concerned a colony

Table 1. *Blood sugar one hour after oral glucose*

	N	15 h fast	2 h fast	6 h fast	*P*
Hyperglycemic SR	15	196 (37)[a]	148 (33)		< 0.01
Normoglycemic SR	8	93 (19)	108 (37)		
White Rats	4	100 (11)	109 (8)		
Hyperglycemic SR	11[b]	187 (32)		176 (30)	
Normoglycemic SR	11[c]	115 (14)		110 (23)	

[a] Figures in parentheses are standard deviations
[b] 11 tests on 7 sand rats
[c] 15 tests on 9 sands rats.

Table 2. *Lesions in arcuate nucleus*

S.R.	Sex	Age (months)	Coordinates (AP)	Lesion	One hour B.S.	Days post lesion	Weights
80A	M	> 6 (estimated)	4.8	Arcuate nuclei	213 (1)[a] / 95 (2)	24	195 (1) / 189 (2)
13	F	> 6 (estimated)	4.8	Periventricular extension of arcuate nuclei and VM nuclei	215 (1) / 96 (2)	14	219 (1) / 209 (2)
107	F	6	4.8	Arcuate nuclei	160 (1) / 128 (1) 1 h 197 (1) / 110 (1) 2 h[b]	45	220 (1) / 215 (1)
84	M	17	4.8	Arcuate nuclei	168 (1) / 111 (1)	23	196 (1) / 182 (1)
45	M	6	4.8	Arcuate nuclei	208 (2) / 107 (1)	24	185 (2) / 203 (1)
89	F	17	4.8	Periventricular extension of arcuate nucleus, arcuate nucleus and VM nucleus	238 (1) / 116 (4)	81	202 (1) / 193 (4)
158	F	6	4.8	Arcuate nucleus (unilateral)	228 (2) / 117 (7)	87	182 (2) / 182 (7)
61	F	6	4.8	Arcuate nucleus (unilateral)	201 (1) / 119 (3); 179 (3) (150) (6)	64 92	209 (1) / 201 (3); 191 (3) (196) (6)
54	F	10	4.8	No lesion ? Electrode tract along 3rd ventricle	125 (1) / 150 (4)	54	271 (1) / 242 (4)

[a] Figures in parenthesis refer to number of determinations.
[b] Peak blood sugar value pre lesion was at 2 h. The pattern of the glucose tolerance remained unchanged post lesion in other animals tested with peak values at 1 h.

It would appear from the present study that lesions of the median eminence, in the area of the arcuate nucleus, lowered the one hour blood sugar in 7 of 9 animals independent of weight change (Table 2). Of the two animals that failed to respond, one animal had a small unilateral lesion in the arcuate nucleus asso-

of wild animals and their offspring, the experimental subjects were of different age, sex and weight. In addition, the size of radio-frequency lesions varies, in our hands at least, perhaps as a result of instantaneous current drop when tissue is destroyed and coats the electrode. Furthermore there is some variability in the

one hour blood sugar in individual sand rats. In order to evaluate this, 40 one hour determinations were performed on 15 hyperglycemic sand rats.

Arbitrarily setting 175 mg% as threshold for hyperglycemic levels and 140 mg% as the upper limit of normoglycemia, 3 of 40 determinations fell to normoglycemic levels. This compares with 3 of 43 in the lesioned group outside the arcuate nucleus and 19 of 39 in the group with lesions of the arcuate nucleus. Since all of the animals were handled in a similar manner, it would appear that lesions involving the arcuate nucleus tend to have a blood sugar lowering effect in the sand rat.

Confirmatory information of a role for the periventricular arcuate system in the diabetic syndrome of these animals will be presented elsewhere [6]. The ventromedial nuclei are in close proximity to and extend alongside the midportion of the arcuate system, which is considered by some to be the final common neuroendocrine path [36]. In a study reported on at this symposium bilateral lesions of the ventromedial nucleus were also associated with a reversion toward normal of hyperglycemic blood sugar values in another diabetic species, the *db* mouse [37].

Our decision to investigate central neurogenic influences on sand rat metabolism was based on earlier studies on growth hormone physiology in other species [2, 10, 8, 28, 30], and also on the response of these animals to fasting and feeding [26, 7]. The mechanism of these neural effects remains to be elucidated.

Table 3. *Lesions outside arcuate nucleus*

S.R.	Sex	Age (months)	Coordinates (AP)	Lesion	One hour B.S.	Days post lesion	Weights
65	F	14	4.8	Mesencephalon	187 (1)[a]/ 197 (4)	87	186 (1) / 155 (4)
50	F	11	4.8	Mammillary bodies	170 (1) / 176 (3)	63	206 (1) / 213 (3)
19	M	12	4.8	Mammillary bodies	181 (1) / 170 (4)	64	218 (1) / 177 (4)
90B	F	14	4.8	Posterior hypothalamus above mammillary bodies	268 (1) / 202 (3)	54	254 (1) / 228 (3)
99B	F	22	4.8	Thalamus at n. reuniens	194 (1) / 189 (3)	51	173 (1) / 172 (3)
67	F	16	4.8	Posterior hypothalamus dorsal to VMN	164 (2) / 163 (3)	26	225 (2) / 195 (3)
23	F	12	4.82	Anterior hypothalamus	211 (2) / 197 (6)	51	158 (2) / 160 (6)
96	F	16	4.89	Anterior hypothalamus	159 (2) / 145 (6)	51	157 (2) / 140 (6)

[a] Figures in parenthesis refer to number of determinations

Acknowledgements. The authors are most grateful to Dr. Harry Hoogstraal (NAMRU-3), the United States Navy, and Dr. K. Schmidt Nielsen for a supply of sand rats. This work was supported in large part by USPHS Grant # 10675 and also # NB 06624.

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