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Effects of Monosodium Glutamate Administration in the Neonatal Period on the Diabetic Syndrome in KK Mice*

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Summary. Administration of monosodium glutamate (MSG) to KK mice during the neonatal period resulted in a syndrome of obesity, stunting and hypogonadism. In some animals the genetic predisposition to diabetes was unmasked with the development of marked hyperglycaemia and or hyperinsulinaemia. Food intake was not increased compared to controls. The elevated plasma glucose and insulin in fed MSG treated mice fell rapidly with food deprivation. Glucose disposal was comparable in MSG treated and control mice after IP glucose, but after oral glucose MSG treated mice showed impaired glucose tolerance. Insulin secretion was defective in MSG treated mice after IP but not after oral glucose.

Key words: Obese mice, genetic diabetes, arcuate nucleus, hypothalamus, hyperinsulinaemia, monosodium glutamate.

Obesity and diabetes commonly coexist although the nature of their inter-relationship is poorly understood. The hypothalamus is known to play an important integrative role in the regulation of food intake and influences carbohydrate and lipid metabolism by a variety of mechanisms, including effects on insulin and glucagon secretion [1]. Evidence for disordered hypothalamic function has been presented in several of the rodent species or strains with spontaneous obesity and or hyperglycaemia [2, 3] and it has been known for many years that destructive lesions of the ventromedial hypothalamic nuclei in rodents give rise to obesity [4]. More recently it has been established that such lesions could result also in hyperglycaemia and hyperinsulinaemia [5, 6]. Further Matsuo & Sino [7] have shown that ventromedial nucleus lesions induced by gold thioglucose (GTG) unmask the diabetic tendency in KK mice. Monosodium glutamate administered subcutaneously to neonatal mice has been reported by Olney [8] to cause destruction of the arcuate hypothalamic nuclei and give rise to a syndrome of obesity, with hypophagia, stunting and infertility. Cameron et al. [9, 10] have shown that such mice are normoglycaemic in the fed state with a tendency to hypoglycaemia after an overnight fast, are insulin sensitive and have mild hyperinsulinaemia. It seemed therefore of interest to examine the effects of such arcuate nucleus lesions on the carbohydrate metabolism of KK mice.

Methods

KK Mice from the Prince Henry's Hospital colony (derived from animals obtained from the Central Institute for Experimental Animals, Tokyo, Japan) were injected subcutaneously with MSG in increasing doses of 2.2–4.4 mg/g from day 1–10 of life [11]. Control mice received an equal volume of normal saline. Mice were weaned at 4 weeks and subsequently fed a commercial chow ad lib.

Animals were weighed at weaning and periodically until sacrificed at 24 weeks. Food intake was assessed periodically by weighing the food administered and subtracting that remaining at the end of defined periods. Preliminary studies had shown that spillage was comparable in both groups. Urine glucose and ketones and plasma glucose and insulin were determined for mice in the fed state 2, 5, 8, 10, 16 and 20 weeks after weaning. At 16 weeks the effect of overnight food deprivation on plasma glucose and insulin

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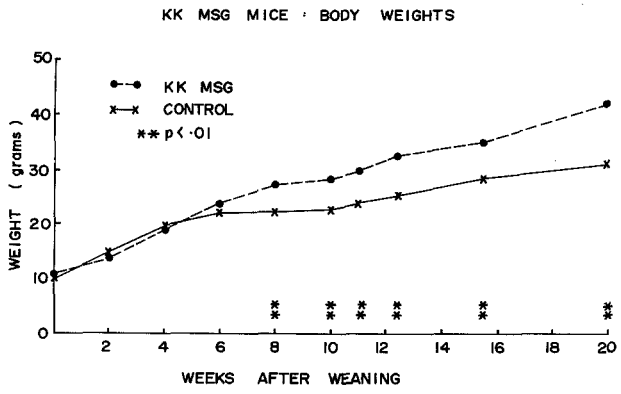

Fig. 1. Body weights of MSG treated and control KK mice from weaning until sacrifice

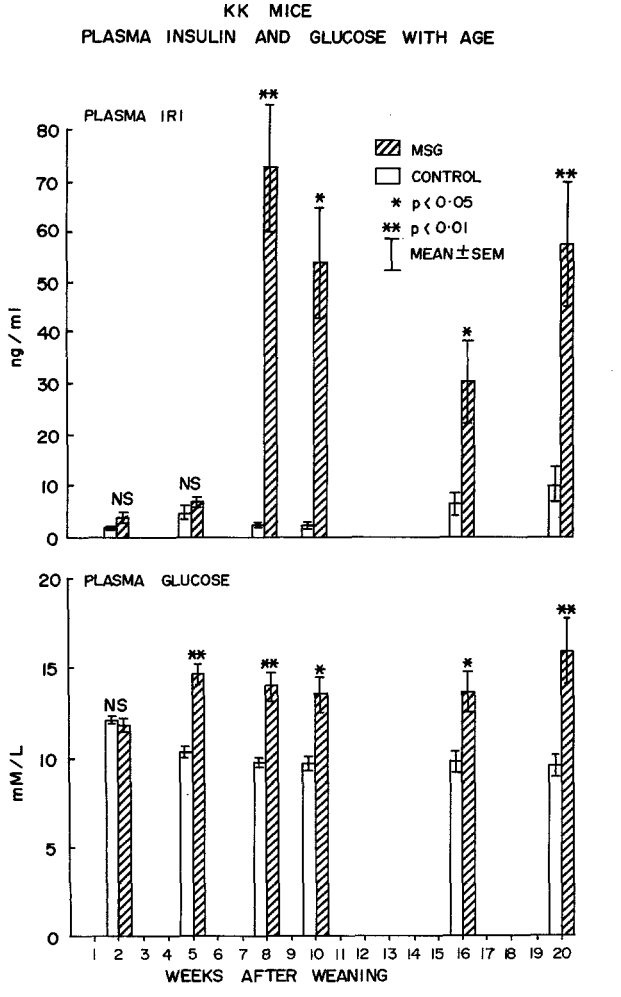

Fig. 2. Plasma glucose and insulin values for MSG treated KK mice and controls in the fed state at various times after weaning. 8–14 control mice were studies at each time and 15–30 MSG

Table 1. Effect of an overnight fast on plasma glucose and insulin

	Fed		Fasted	
	Glucose mMol/L	Insulin ng/ml	Glucose mMol/L	Insulin ng/ml
KK MSG (n=23)	13.62±1.22	30.53±8.2	5.2±0.43	2.66±0.64
	P<0.05	p<0.05	N.S.	p<0.05
Control (n–10)	9.73±0.6	6.22±2.0	4.08±0.38	0.92±0.14

Means ± SEM Number of animals in parenthesis

was examined. The effect of intraperitoneal (IP) and oral glucose administration (1.0 g/kg) was studied in different groups of mice fasted overnight and anaesthetised with pentobarbitone sodium 60 mg/kg. Blood samples were obtained at 0, 2, 5, 15 and 45 min in the I.P. study and 0, 15, 30 and 60 min in the oral. Insulin sensitivity was assessed by the administration of regular insulin 0.075 u/kg intravenously to mice in the fed state and after an overnight fast. Blood samples were obtained at 0, 5, 10, 20 and 30 min. Blood samples in all studies were taken from the orbital venous plexus in heparinised capillary tubes, transferred immediately to chilled, heparinised Microfuge tubes and the plasma separated by centrifugation in a Microfuge (Beckman Instruments). Plasma was stored at −20 °C until assayed. Mice were sacrificed in the fed state and testicular and pituitary weights determined. Brains of six representative MSG treated and six control animals were taken for histological examination. These were fixed in 10% neutral formalin, serial 10 μ coronal sections were taken through the hypothalamus and stained with toluidine blue. Pancreases were extracted by sonication with a Branson Sonicator Model B30 in 1N acetic acid pH 2.4 for determination of insulin content. Urine glucose was estimated with Clinistix (Ames) and urine ketones with Ketostix (Ames). Plasma glucose was determined by a glucose oxidase method in 5 μl samples and plasma and pancreatic insulin by radioimmunoassay using a mouse insulin standard (Lot M 181072 Novo Industri A/S) as described previously [12]. Statistical comparisons between groups were carried out using Student's t test.

Results

Figure 1 shows the body weights of MSG treated and control mice from weaning until sacrifice. It is evident that MSG treated mice were significantly heavier than controls by eight weeks after weaning and subsequently became markedly obese. MSG mice were stunted compared to controls. At 20 weeks postwean-

ing the nasoanal length of MSG mice was 8.94 ± 0.1 cm (n = 14) mean ± SEM and of control mice 9.61 ± 0.13 cm (n = 14) $p < 0.01$. The Lee Index $\left(\frac{\sqrt[3]{\text{Weight (g)}}}{\text{Length} \cdot \text{(cm)}} \times 10^3\right)$, a measure of the degree of adiposity [13] was significantly greater ($p<0.01$) in MSG mice, 393.1 ± 2.8 (n = 14), than controls, 320.4 ± 5.4 (n = 14), (mean ± SEM) at 20 weeks after weaning. Food intake was not significantly different between the two groups during the eight weeks after weaning-MSG 3.1 ± 0.1 g/mouse/day and Control 3.39 ± 0.13 g/mouse/day (mean ± SEM). Commencing 2 weeks after weaning 2 out of 31 MSG treated mice showed 2% glycosuria and the number of glycosuric animals increased with time so that from 8 weeks after weaning until sacrifice at 20 weeks approximately 40% of the treated animals had 2% urinary glucose. No control KK mice showed more than a trace of urinary glucose. Ketonuria was not observed in any animals.

Figure 2 summarises the plasma glucose and insulin levels in fed MSG and control mice at various times after weaning. No significant difference in plasma glucose was present at two weeks, but subsequently MSG treated mice consistently had a significant elevation of plasma glucose. Plasma insulin levels, while tending to be higher in MSG treated mice at 2 and 5 weeks after weaning, were markedly and significantly elevated at subsequent times. Considerable variability in the levels of plasma insulin was observed in the same mice at different times, whereas the plasma glucose levels remained comparable in the same mice from the 5th week onwards.

In Table 1 plasma glucose and insulin in mice 16 weeks after weaning in the fed state and after an overnight fast are shown. Plasma glucose fell in both groups with fasting, but to a much greater extent in MSG treated than control mice such that, at this time, there was no significant difference between the groups. Plasma insulin also fell in both groups. The fall was again greater in MSG treated animals; however, the levels were still significantly ($p<0.05$) greater than in controls.

The effect of IP glucose administration is shown in *Figure 3*. Plasma glucose levels were not significantly different between the two groups at any time. Plasma insulin levels, however, differed markedly in that the peak seen at 2 min in the control mice was not noted in the MSG treated animals. With oral glucose administration *(Fig. 4)* glucose disposal was significantly impaired in MSG mice compared to the controls despite higher plasma insulin levels. *Figure 5* shows the plasma glucose response to I.V. insulin in fed and fasted KK mice. In both fed and fasted states the fall in plasma glucose was less in MSG treated than con-

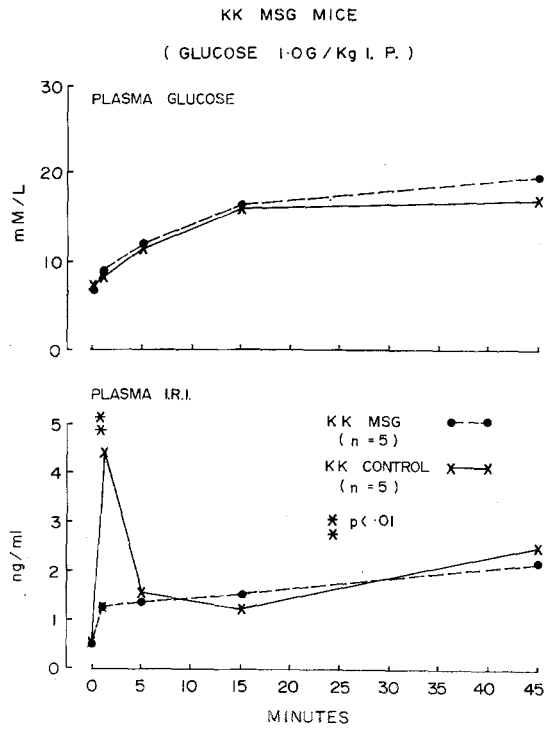

Fig. 3. MSG treated and control KK mice. Effect of intraperitoneal glucose 1.0 g/kg on plasma glucose and insulin. Animals were fasted overnight before the test

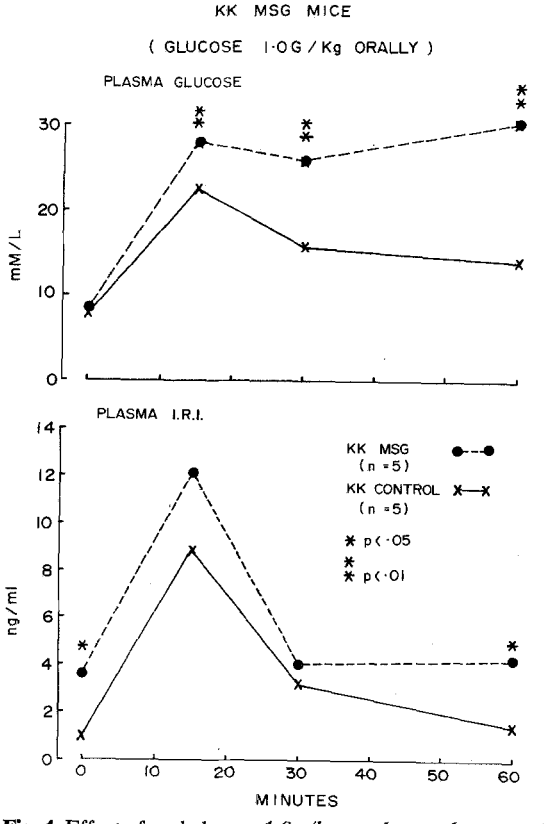

Fig. 4. Effect of oral glucose 1.0 g/kg on plasma glucose and insulin in MSG treated •---• and control ×——× KK mice after an overnight fast

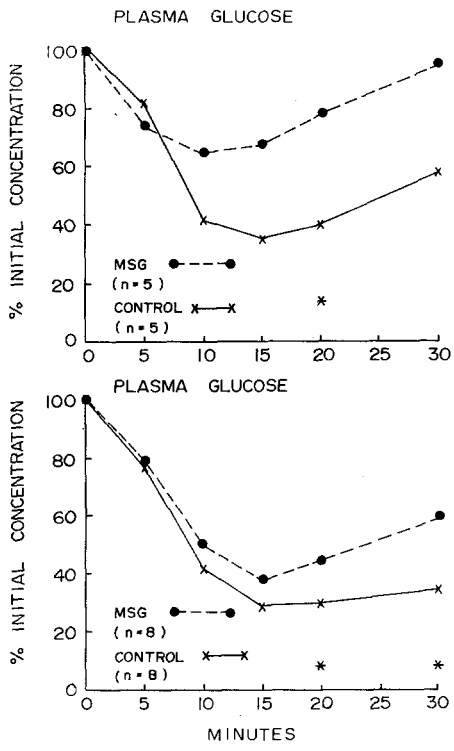

Fig. 5. Effect of insulin 0.075/kg I.V. on plasma glucose in the MSG treated and control KK mice. In the upper panel data from animals studied in the fed state are shown in the lower panel from animals studied after an overnight fast. All values are presented as a percentage of the initial value. MSG treated were compared with control at each time point *$p<0.05$

Table 2. Insulin concentration and content of KK mice killed 20 weeks after weaning

	Pancreatic insulin concentration ng/mg wet weight	Total pancreatic insulin μg/pancreas
Control (n=7)	93.2 ± 16.97	23.83 ± 3.95
	N.S.	N.S.
MSG (n=14)	112.4 ± 24.37	23.14 ± 5.46

Mean ± SEM Number of animals in parenthesis

trol mice and the return towards basal was more prompt.

No significant difference in pancreatic insulin content or concentration was seen between the two groups of mice sacrificed at 20 weeks (Table 2). Table 3 lists morphometric characteristics of mice killed 20 weeks after weaning. MSG treated mice showed significant diminution of pituitary and testicular weight. Histological examination of the hypothalamus of the MSG treated and control mice showed lesions in the arcuate nuclei of the MSG treated animals with loss of neuronal cell bodies and collapse similar to that described for normal mice treated with MSG [10].

Discussion

The KK strain of mice is an inbred strain with a genetic predisposition to diabetes. Overt diabetes, however, does not usually occur when the mice are kept under standard laboratory conditions [14, 15, 16]. In our colony only occasional animals exhibit glycosuria. It has been shown that diabetes in KK mice can be unmasked when they are fed a high carbohydrate diet [14] or when made obese either by the introduction of the A^y gene [15] or by gold thioglucose induced ventromedial nucleus hyperphagia. The latter treatment unmasks the diabetic tendency in approximately 50% of the treated animals. Considerable differences were, however, noted in the metabolic state of the mice treated with MSG between the fed and fasted states. In the fed state the treated animals as a group were hyperglycaemic, hyperinsulinaemic and insulin resistant. However, after an overnight fast, plasma glucose and insulin values were comparable in both treated and control mice. In the fasted state the mice given MSG continued to show a degree of insulin resistance, were glucose intolerant after an oral glucose load and failed to release insulin rapidly after intraperitoneal glucose administration.

The aetiology of obesity in MSG treated mice is still uncertain but it is clearly not the result of hyper-

Table 3. Morphometric characteristics of KK mice killed at 20 weeks after weaning

	Weight g	Length cm	Lee index	Pituitary weight mg	Testicular weight mg
Control	29.6 ± 1.3 (n = 14) **	9.61 ± 0.13 (n = 14) **	320.4 ± 2.8 (n = 14) **	1.76 ± 0.13 (n = 13) **	202.4 ± 11.1 (n = 6) **
MSG	43.6 ± 1.26 (n = 14)	8.94 ± 0.1 (n = 14)	393.1 ± 5.4 (n = 14)	0.64 ± 0.09 (n = 12)	95.4 ± 31.8 (n = 6)

Mean ± SEM Number of animals in parenthesis

** $p<0.01$

phagia either in this study or in those on normal mice given MSG [8, 9, 10, 17]. Difficult to reconcile with this are the findings of Araujo and Mayer [17] who, using a tilt table, reported increased activity in MSG treated normal mice compared to their controls. No estimate of energy expenditure was undertaken in the present study and further detailed examination of the pattern of food intake in treated animals and of energy expenditure appear indicated. Han et al. [18] and Bernardis & Skelton [19, 20] have shown that weanling rats with electrolytic lesions in the ventromedial hypothalamic nuclei became obese without excessive caloric intake. Frohman and Bernardis [6] concluded that at least one major factor contributing to excessive accumulation of adipose tissue in VMN lesioned weanling rats was growth hormone deficiency resulting from the hypothalamic lesions. The arcuate nucleus is known to be a new area for the regulation of growth hormone secretion [21] and growth hormone deficiency seems likely in the MSG treated mice in the present study in view of the stunting and decreased pituitary weights. However, massive obesity of the type seen in the mice given MSG is not a usual consequence of hypophysectomy. That the hypothalamus may affect lipolysis directly via the autonomic nervous system has been proposed by Goodner [22]. The possibility that impaired regulation of lipolysis by the autonomic nervous system resulting from the arcuate nucleus destruction may contribute to the obesity in MSG treated mice requires study. Hyperinsulinaemia, when present, is doubtless of importance in directing ingested calories to fat storage but is unlikely to be of primary aetiological significance in the present study since, while obesity was a constant finding, hyperinsulinaemia was not.

The cause of hyperglycaemia and hyperinsulinaemia in treated mice is not certain. While all MSG treated KK mice became obese not all became diabetic or hyperinsulinaemic and no significant correlation was found between plasma glucose or plasma insulin levels and body weight or adiposity as measured by the Lee Index. In the studies of Iwatsuka et al. in KK mice carrying A^y gene [15] and of Matsuo and Shino in KK mice with gold thioglucose induced obesity [7] hyperglycaemia was considered to stem from obesity causing insulin resistance. As in the above studies and in contrast to observations in normal mice treated with MSG [10], KK MSG mice showed a degree of insulin resistance. Insulin resistance, however, was present also in the fasted state when MSG treated mice did not exhibit hyperglycaemia and when plasma insulin had fallen to levels approaching those in controls. The hyperglycaemia in KK mice treated with MSG appears to be very dependent on food intake as evidenced by the marked fall in plasma glucose in MSG treated mice compared with controls when this was given by the oral rather than the I.P. route. It is clear from the studies of Frohman [23] & Shimazu [24, 25] that the hypothalamus may influence hepatic glucose output and Szabo & Szabo [26] have recently demonstrated an intracranial insulin sensor which effects a rapid fall in plasma glucose via the autonomic nervous system. One may postulate that MSG treatment has disturbed such regulatory mechanisms resulting in hyperglycaemia; however, the difference in the effects on blood glucose between MSG treated normal and KK mice remains unexplained. As with hyperglycaemia the elevated plasma insulin levels in the MSG treated KK mice do not appear to be a direct consequence of the obesity, the degree of hyperinsulinaemia appears to be clearly related to oral substrate intake. Studies in ventromedial nucleus lesioned animals have shown that at least part of the hyperinsulinaemia which occurs is independent of hyperphagia and may be a direct consequence of the hypothalamic lesion [27, 29]. No comparable studies have been undertaken in MSG treated animals. The finding of comparable pancreatic insulin content and concentration was somewhat surprising in view of the marked hyperinsulinaemia present in some animals. V. M. N. lesions in KK mice have been shown to cause islet hyperplasia with degranulated B cells [7]. No histological examinations were carried out to assess the size of the islet mass in the present study or the degree of granulation of B cells.

This study, therefore, shows that MSG induced arcuate nucleus lesions produce a variety of metabolic abnormalities and may unmask the genetic tendency to diabetes in this strain. The mechanisms responsible for hyperglycaemia and hyperinsulinaemia are not clear. Obesity and insulin resistance do not appear to be primarily responsible and one may postulate a more direct role of the hypothalamic lesion. The reason why only some treated mice developed hyperglycaemia or hyperinsulinaemia may be related to the extent of the lesion produced by MSG. Bernardis & Frohman [29] have shown that the extent of the electrolytic lesions produced in the region of the ventromedial nuclei of weanling rate affected the levels of growth hormone and insulin in plasma. No systematic study of lesion size was made in the present group of animals although in six representative mice it was confirmed that the lesions were located in the arcuate nuclei. Further, the factors responsible for hyperglycaemia and gross hyperinsulinaemia in MSG treated KK mice, as compared to similarly treated normal mice, are unclear and, no doubt, reflect unidentified genetic factors operating in this strain. This model deserves further study in an attempt to gain greater insight into the relationship of obesity to dia-

betes and to the possible role of the hypothalamus in modulating this relationship.

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References

1. Frohman, L. A.: The hypothalamus and metabolic control. Pathobiology Annual **1**, 353–372 (1971)
2. Bray, G. A., York, D. A.: Genetically transmitted obesity in rodents Physiol. Rev. **51**, 598–646 (1971)
3. Cameron, D., Stauffacher, W., Renold, A. E.: Spontaneous hyperglycaemia and obesity in laboratory rodents. In: Handbook of physiology, endocrine pancreas (eds. D. F. Steiner, Freinkel), pp. 611–625. Washington: American Physiology Society 1972
4. Hetherington, A. W., Ranson, S. W.: Hypothalamic lesions and adiposity in the rat. Anat. Rec. **78**, 149–172 (1940)
5. Hales, C. N., Kennedy, G. C.: Plasma glucose, non-esterified fatty acids and insulin concentrations in hypothalamic-hyperphagic rats. Biochem. J. **90**, 620–624 (1964)
6. Frohman, L. A., Bernardis, L. L.: Growth hormone and insulin levels in weanling rats with ventromedial hypothalamic lesions. Endocrinology **82**, 1125–1132 (1968)
7. Matsuo, T., Shino, A.: Induction of diabetic alterations by gold thioglucose obesity in KK, ICR and C57 BC mice. Diabetologia **8**, 391–397 (1972)
8. Olney, J. W.: Brain lesions, obesity and other disturbances in mice treated with monosodium glutamate. Science **164**, 719–721 (1969)
9. Cameron, D. P., Cutbush, L., Opat, F.: Monosodium glutamate induced obesity in mice: Studies on plasma glucose and immunoreactive insulin. In: Recent advances in obesity research 1. (ed. A. N. Howard), pp. 134–137. London: Newman Publishing 1975
10. Cameron, D. P., Cutbush, L., Opat, F.: Plasma glucose and insulin in monosodium glutamate induced obesity in mice. Clin. Exp. Pharm. Phys. (In press)
11. Potts, A. M., Modrell, K. W., Kingsburg, C.: Permanent fractionation of the electroretinogram by sodium glutamate. Amer. J. Ophthal. **59**, 900–907 (1960)
12. Cameron, D. P., Stauffacher, W., Amherdt, M., Orci, L., Renold, A. E.: Kinetics of immunoreactive insulin release in obese hyperglycaemic laboratory rodents. Endocrinology **92**, 257–264 (1973)
13. Bernardis, L. L., Patterson, B. D.: Correlation between 'Lee Index' and carcass fat in weanling and adult female rats with hypothalamic lesions. J. Endocr. **40**, 527–528 (1968)
14. Matsuo, T., Shino, A., Iwatsuka, H., Suzuoki, Z.: Induction of overt diabetes in KK mice by dietary means. Endocr. jap. **17**, 477–488 (1970)
15. Iwatsuka, H., Shino, A., Suzuoki, Z.: General survey of diabetic features of yellow KK mice. Endocr. jap. **17**, 23–35 (1970)
16. Matsuo, T., Shino, A., Iwatsuka, H., Suzuoki, Z.: Studies on diabetogenic action of obesity in mice. Congenital insulin resistance of KK mice. Endocr. jap. **17**, 535–540 (1970)
17. Araujo, P. E., Mayer, J.: Activity increase associated with obesity induced by monosodium glutamate in mice. Amer. J. Physiol. **225**, 764–765 (1973)
18. Han, P. W., Lin, G.-H., Chu, K.-C., Mu, J.-Y., Liu, A.-C.: Hypothalamic obesity in weanling rats. Amer. J. Physiol. **209**, 627–631 (1965)
19. Bernardis, L. L., Skelton, F. R.: Growth and obesity following ventromedial hypothalamic lesions placed in female rats at four different ages. Neuroendocrinology **1**, 265–275 (1965)
20. Bernardis, L. L., Skelton, F. R.: Growth and obesity in male rats after placement of ventromedial hypothalamic lesions at four different ages. J. Endocr. **38**, 351–352 (1967)
21. Martin, J. B.: Neural regulation of growth hormone secretion. New Engl. J. Med. **288**, 1384–1393 (1973)
22. Goodner, C. J., Tustison, W. A., Davidson, M. B., Chu, P.-C., Conway, M. J.: Studies of substrate regulation in fasting. 1. Evidence for central regulation of lipolysis by plasma glucose mediated by the sympathetic nervous system. Diabetes **16**, 576–589 (1967)
33. Frohman, L. A., Bernardis, L. L.: Effect of hypothalamic stimulation on plasma glucose, insulin and glucagon levels. Amer. J. Physiol. **221**, 1596–1603 (1971)
24. Shimazu, T., Fukuda, A., Ban, T.: Reciprocal influences of ventromedial and lateral hypothalamic nuclei on blood glucose level and liver glycogen content. Nature (Lond.) **210**, 1178–1179 (1966)
25. Shimazu, T., Amakawa, A.: Regulation of glycogen metabolism in liver by the autonomic nervous system 11. Neural control of glycogenolytic enzymes. Biochim. biophys. Acta. (Amst.) **165**, 335–348 (1968)
26. Szabo, O., Szabo, A. J.: Evidence for an insulin sensitive receptor in the central nervous system. Amer. J. Physiol. **223**, 1349–1353 (1972)
27. Han, P. W., Frohman, L. A.: Hyperinsulinaemia in tube-fed hypophysectomised rats bearing hypothalamic lesions. Amer. J. Physiol. **219**, 1632–1636 (1970)
28. Stauffacher, W., Orci, L., Marliss, E., Cameron, D. P.: Nutritional influences on hyperglycaemic syndromes in laboratory rodents. Suggested usefulness of an "under developed" area of research. Acta diabet. lat. **9**, 579–596 (1972)
29. Bernardis, L. L., Frohman, L. A.: Effect of lesion size in the ventromedial hypothalamus on growth hormone and insulin levels in weanling rats. Neuroendocrinology **6**, 319–328 (1970)

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