halothane for positive identification of the susceptible animals. Serum CPK activity was assayecd by the coupled enzymatic method using the 'Monotest' CPK kit supplied by Boehringer GMBH, Mannheim, West Germany, and expressed as International Units per litre of serum (IU/l) at 30 °C.

Results and Conclusions. The Figure shows the variation of serum CPK activity with age for the 7 halothane-resistant and 3 halothane-susceptible pigs. CPK levels reached a peak at 19 weeks of age in both groups, and after 28 weeks the levels returned to values similar to those at 11 weeks of age. The 'normal' serum CPK activities were 217.3 ± 23.2 IU/l and 254.5 ± 24.5 IU/l (mean ± S.E.M.) at 11 and 28 weeks of age, respectively. There was no sex difference in serum CPK activity at any age. There were no statistically significant differences (p 0.05) between the CPK activities of the resistant and susceptible pigs at 15, 19 and 23 weeks of age, but significant differences were obtained at 11 and 28 weeks of age ($p < 0.01$; Student's t-test). These findings strongly indicate that age is a major factor determining serum CPK levels in both normal and MH susceptible pigs alike. In the group of pigs studied serum CPK activity reached a peak value at 19 weeks of age, an age correlating well with the period of maximum growth rate[9] and more specifically with the process of 'muscling'[10]. According to Hammond[10], 'muscling' refers to the period of maximal muscle protein anabolism and increase in muscle fibre size. We therefore infer that the increase in serum CPK activity observed during the 15th to 23rd week of age in the present study is directly related to the 'muscling' phenomenon. The increase in serum enzyme activity may be interpreted as an increase in the leakiness of the skeletal muscle fibres during the period of maximal muscle growth. The fact that the serum CPK levels in the MH susceptible pigs are significantly elevated at 11 and 28 weeks of age compared with the non-susceptible animals is in agreement with the increases in CPK activity observed in DMD and other myopathies[1] and in the human syndrome[2].

From these observations it is clear that there are numerous factors causing non specific increases in serum CPK levels. However, in the group of pigs studied, after minimizing these extraneous factors, we were able to detect those animals carrying the MH syndrome on the basis of significantly elevated serum CPK levels.

Zusammenfassung. Infolge Muskeltätigkeit steigt bekanntlich der Creatin-Phosphokinase-Spiegel (CPK) im Serum. Es wird nun eine Abhängigkeit des Serum-CPK-Wertes vom Alter festgestellt. Es ist daher wichtig, nichtspezifische Ursachen eines gesteigerten CPK-Spiegels auszuschalten, ehe eine Diagnose der Muskelerkrankung gestellt wird.

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[9] J. HAMMOND, in *Farm Animals* (Edward Arnold, London 1965), p. 158.

[10] J. HAMMOND, quoted in *Animal Nutrition* (Eds. L. A. MAYNARD and J. K. LOOSLI; McGraw-Hill, New York 1962), p. 378.

[11] We thank R. T. NAUDE for advice. This work was supported by the Atomic Energy Board of South Africa and the Senate of the University of the Witwatersrand.

Gastrointestinal Hormones and Neural Interaction Within the Central Nervous System

Obesity is one of the most common nutritional disorders in the world effecting millions of overweight people. Obesity is a major contributor to heart diseases. The underlying cause of obesity is unknown. Therapy of this disorder is generally unsatisfactory despite modern medical use of various drugs, operations and the popularity of fad diets. The basic problem for the fat person is that his appetite is not satisfied until he has consumed too much food.

BEAUMONT[1] noted that St. Martin required instillation of food into the upper gastrointestinal tract to experience satiety from hunger. Parenteral injection of intestinal mucosal extracts, presumably containing soluble polypeptide hormones, caused depression of hunger and weight loss in rabbits[2]. GIBBS et al.[3,4] injected unfed rats with purified cholecystokinin (CCK) or the synthetic octapeptide (CCK-OP) which possesses all biological activity of the entire CCK molecule; these agents evoked the satiety response. Other gastrointestinal hormones (pentagastrin and secretin) did not stimulate the satiety response in the rats. FARA et al.[5] noted that CCK induced somnolence in cats. Satiety was evoked by feeding, which is also known to produce release of gastrointestinal hormones, including CCK[6]. CCK is released normally when the upper small intestinal mucosa is exposed to ingested fat, amino acids and gastric juice[7]. The specific target areas of a satiety factor probably lie within the hypothalamus, since different lesions of the region can instigate or repress hunger[8,9].

The aim of this investigation was to determine electrophysiological response of various areas of the brain to the hormonal satiety factor. Central effects of CCK or other gastrointestinal hormones can be expected to result from modulation of neuronal activities, and such actions may be localized in one or more brain structures. The present study represents an initial attempt to elucidate the synaptic effects of gastrointestinal hormones in several structures within the brain which are presumably involved in controlling and regulating the appetite.

[1] W. BEAUMONT, *Experiments and Observations on the Gastric Juice and Physiology of Digestion* (Dover Publications, New York 1959), p. 208.

[2] N. F. MACLAGAN, J. Physiol., Lond. *90*, 385 (1937).

[3] J. GIBBS, R. C. YOUNG and G. P. SMITH, J. comp. Physiol. Psych. *84*, 488 (1973).

[4] J. GIBBS, R. C. YOUNG and G. P. SMITH, Nature, Lond. *245*, 323 (1973).

[5] J. W. FARA, E. H. RUBINSTEIN and R. R. SONNENSCHEIN, Science *166*, 110 (1969).

[6] C. C. WANG and M. I. GROSSMAN, Am. J. Physiol. *164*, 527 (1951).

[7] L. R. JOHNSON, in *International Review of Physiology, Gastrointestinal Physiology* (Eds. E. D. JACOBSON and L. L. SHANBOUR; MTP, Oxford 1974), p. 1–44.

[8] B. G. HOEBEL and P. TEITELBAUM, J. comp. Physiol. Psychol. *21*, 189 (1966).

[9] T. L. POWLEY and R. E. KEESY, J. comp. Physiol. Psychol. *70*, 25 (1970).

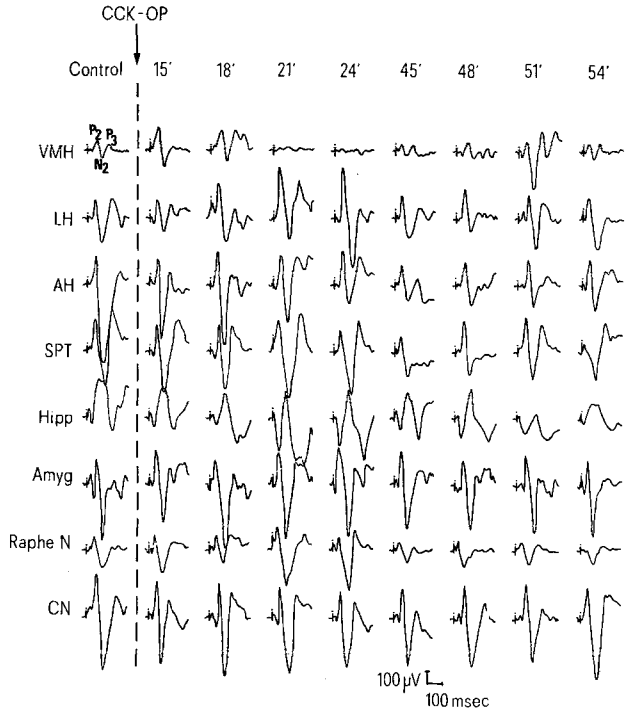

Fig. 1. Average evoked responses to click stimuli recorded from ventromedial hypothalamus (VMH), lateral hypothalamus (LH), anterior hypothalamus (AH), septum pellucidum (SPT), anterior hippocampus (Hipp), amygdalla complex (Amyg), raphe nucleus (Raphe N), and caudate nucleus (CN). Each trace consists of the responses to 32 consecutive click stimuli prior to drug injection (Control) and the responses following i.p. administration of the synthetic octapeptide (CCK-OP) of cholecystokinin. The numbers indicate time in min post injection.

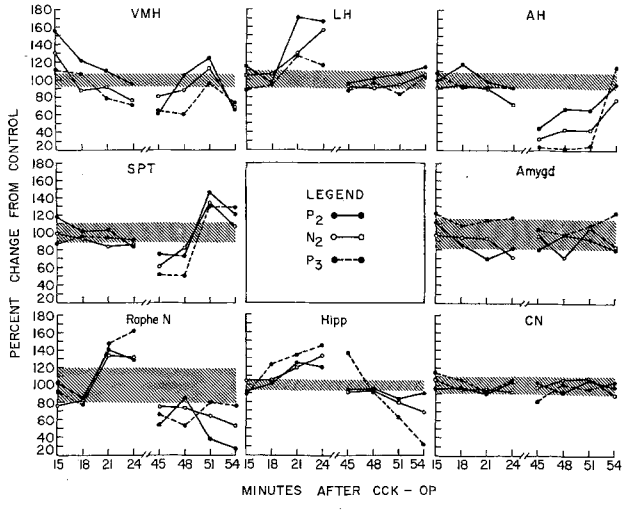

Fig. 2. Average direction of responsiveness from animals expressed as a percentage of control. The control recordings are 100%, the shaded area expresses the variation in the control recording. Values greater than or less than 100% express an increase or decrease in amplitude in comparison with control amplitude of each of the 3 late components of the responses recorded from VMH, LH, AH, SPT, Amyg, Raphe N, Hipp and CN.

[10] J. F. R. König and R. A. Klippel, *A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem* (Williams and Wilkins, Baltimore, Md. 1973).

Responses evoked by click (acoustic) stimuli were recorded simultaneously from the ventromedial hypothalamus (VMH), lateral hypothalsmus (LH), anterior hypothalamus (AH), septum pellucidum (SPT), dorsal hippocampus (Hipp), amygdala complex (Amyg), raphe nucleus (Raphe N), and caudate nucleus (CN) in the awake, freely moving rat. The responses were recorded monopolarly with chronically implanted nichrome electrodes of 50 μm diameter and a reference electrode implanted in the frontal sinuses. 1 week before experimentation electrodes were placed in anesthetized rats using sterotaxic coordinates derived from the atlas of König and Klippel[10], and the placement was verified histologically in all animals. The evoked potentials were displayed on a storage oscilloscope, average on line with NIC 1070 computer and recorded with an X-Yplotter.

The experiments were carried out on 18 Holtzman male rats weighing 250–350 g, divided into 2 groups: 1. those which received pentagastrin (100 μg/kg) followed by synthetic secretin (1 μg/kg); and 2. those which were injected i.p. with CCK-OP (1 μg/kg). Each animal served as its own control; that is, control recordings were taken prior to the administration of each hormone. Each experiment consisted of: 1. an initial period of 4 h of adaptation of the animal to the experimental setting; 2. 1 h of control recording taken every 10 min; 3. administration of the hormone (gastrin or CCK) followed by recording at every 3 min, starting 15 min postinjection; 4. administration of the second hormone (secretin) or a second set of recordings after the first set, again with recordings obtained 15 min after injection. Each recording consisted of the average field potentials evoked by 32 consecutive click stimuli at 2.5 sec.

The configuration of the averaged acoustic evoked response (AAER) consisted of an initial diphasic (positive-negative) low amplitude spike succeeded by a large triphasic (positive-negative-positive) wave. These components were labeled as P_1, N_1, P_2, N_2 and P_3 respectively. Figure 1 (left column) illustrates the characteristic AAER recorded in 8 structures. Neither pentagastrin nor synthetic secretin modified the AAER in any of 8 recording sites. However, in the majority of areas CCK-OP altered the AAER with most pronounced effects in the hypothalamus and limbic systems, with the exception of the Amyg and CN.

The findings of major interest in the present experiments are: 1. CCK-OP caused an early transient increase in electrical activity in the VMH followed by a prolonged decreased in the AAER which recovered after 50 min; 2. in the LH, CCK-OP caused an increase in the AAER at 21–24 min postinjection; 3. the AAER was attenuated in the AH from 45 to 60 min after injection of CCK-OP; 4. in the SPT, Hipp and Raphe N mixed responses were recorded, and in the Amyg and CN no alteration in the AAER was observed (Figure 1). Figure 2 is a calculated summary of all the rats injected with CCK-OP. Components P_2, N_2 and P_3 of the control AAER have been converted to 100%, the fluctuation in the control recordings are shaded and separation is depicted on the basis of the direction of change, i.e., increase or decrease. The average of this group of animals are similar to those observed in individual specimens.

Gross electrodes were employed in these investigations to make the responses more apparent and to provide average sampling of sizeable neuronal populations. This approach combines the convenience of time-lock in the test procedure with recording of many neuronal units in parallel. Areas of the central nervous system not ordinarily considered part of the 'auditory system' (i.e., hypothalamus, limbic system, reticular formation and basal ganglia)

exhibit responses to acoustic stimuli that are not readily distinguished from responses observed in classical auditory pathways[11–15]. The physiological sensory input we used, acoustic stimulation, served as a tool to initiate synaptic activities in neural structures which may control appetite as well as in other structures not apparently related to appetite. The doses of gastrointestinal hormones we administered are doses which drive their respective normal target organs maximally in the rat. They are doses which are likely to be encountered in the body under normal conditions. In conclusion, our findings suggest that there may be a basis for the concept that the gastrointestinal tract can modulate central neuronal activity during feeding by release of a hormone, CCK. Injection of CCK-OP in conscious rats modified the AAER in several structures, notably areas of the hypothalamus linked to control of appetite.

Zusammenfassung. Bei Ratten wurde die Tätigkeit des ZNS mit Hilfe von in diverse Hirnzonen implantierten Elektroden registriert, wobei die sensorische Reizung im Wachzustand zur Aktivierung der elektrischen Aktivität benutzt wurde. Injektionen von Cholecystokinin Oktapeptid in Zonen mit Appetitregulation führte zu systematischer Veränderung der durchschnittlichen Reizbeantwortung.

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[11] D. ALBE-FESSARD, E. OSWALDO and C. ROCHA-MIRANDA, Electroenceph. clin. Neurophysiol. *12*, 405 (1960).
[12] N. DAFNY, Electroenceph. clin. Neurophysiol. *36*, 123 (1974).
[13] R. GALAMBOS, *Neural Mechanisms of the Auditory and Vestibular System* (Eds. G. L. RASMUSSEN and W. F. WINDLE; Thomas, Springfield, Ill. 1959), p. 137.
[14] B. L. JACOBS and M. J. MCGINTY, Brain Res. *36*, 431 (1972).
[15] R. F. THOMPSON, H. F. SMITH and D. BLISS, J. Neurophysiol. *23*, 365 (1963).

Increased Erythrocytes Sodium Efflux in Genetic Hypertensive Rats of the Hebrew University Strain

Several investigators have attempted to establish a correlation between hypertension and an abnormal intracellular sodium distribution. Increased sodium content in the arterial wall has been reported in hypertensive subjects[1] and in various types of experimental hypertension[2–4]. Increased sodium content has been also found in the erythrocytes of hypertensive subjects and their normotensive relatives[5,6] and more recently in the leucocytes of hypertensive subjects[7]. In one study, the high erythrocyte sodium concentration was attributed to an increased sodium influx[6]. Recent studies from this laboratory have shown an increased efflux of Na^{22} from erythrocytes of subjects with uncomplicated essential hypertension[8]. These studies have now been extended to experimental hypertension in rats.

We have recently developed in this laboratory 2 strains of rats with markedly dissimilar susceptibility to Doca-salt hypertension[9]. The hypertension-prone (H) rat invariably develops hypertension on Doca-salt treatment whereas the normotensive (N) rat maintains normal blood pressure on the same regimen. The H rat also tends to develop mild spontaneous hypertension under regular laboratory conditions. The results presented here show an increased erythrocyte sodium efflux in the hypertension-prone rats.

Materials and methods. Male rats from the hypertension-prone (H) and hypertension resistant (N) strains developed in this laboratory were used[9]. The animals were kept in an artificially illuminated environment and maintained on regular laboratory chow and tap water. All experiments were run in pairs of H and N rats of approximately comparable age and weight. In the H group we have chosen rats with the highest levels of spontaneous hypertension. None of the animals had been subjected to any manipulation prior to this study. Systolic blood pressure was determined by the tail microphonic method of FRIEDMAN and FRIED.

Sodium efflux was studied by a modification of the methods of SACHS and WELT[10] and GARDNER et al.[11]. Heparinized blood was obtained by cardiac puncture under light ether anesthesia. The plasma and buffy coat were removed after centrifugation at 3000 rpm for 5 min at room temperature. Erythrocytes were washed three times with chilled isosmotic $MgCl_2$ and suspended in a ratio of 1:3 in a solution containing 10% sodium phosphate buffer (pH 7.4), 90% isosmotic sodium chloride and 500 mg/100 ml glucose. Approximately 10 μCi of Na^{22} were added and the suspension incubated in a rotor for 3 h at 37 °C. The cells were separated by centrifugation and washed 3 times with iced isosmotic $MgCl_2$ solution containing 10 m*M tris* buffer (pH 7.4). The erythrocytes were resuspended in an incubation medium at a hematocrit of 3–5%. The medium had the following composition (m*M*): *Tris* buffer (pH 7.4), 23; NaCl, 146; KCl 6; KH_2PO_4, 0.3. After thorough mixing, 2 samples of 2 ml each were taken from the suspension at time zero. In one sample, the cells were lysed with saponin, and, after thorough mixing, 1 ml of the hemolysate was counted. The second sample was chilled in crushed ice for 2–3 min centrifuged and 1 ml of the supernatant transferred into a counting vial. The suspension of cells was placed in an oscillating water bath at 37 °C and additional 2 ml samples were obtained at 15, 30, 45, 60 and 75 min. Each sample was chilled, centrifuged and 1 ml of the supernatant removed for counting as above. Radioactivity was measured with a Packard model 3004 liquid scintillation spectrometer.

[1] L. TOBIAN JR. and J. T. BINION, Circulation *5*, 754 (1952).
[2] L. TOBIAN JR. and P. REDLEAF, Am. J. Physiol. *192*, 325 (1958).
[3] E. L. PHELAN and L. C. K. WONG, Clin. Sic. *35*, 487 (1968).
[4] A. NAGAOKA, K. KIKUCHI and Y. ARAMAKI, Jap. Circulation J. *34*, 489 (1970).
[5] U. GESSLER, Z. Kreislaufforsch. *51*, 177 (1962).
[6] F. WESSELS, G. JUNGE-HULSING and H. LOSSE, Z. Kreislaufforsch. *56*, 374 (1967).
[7] R. D. THOMAS, R. P. S. EDMONDSON, P. J. HILTON and N. P. JONES, Third Meeting of the International Society of Hypertension Milan (1974).
[8] A. AVIRAM, Proc. 5th International Congress of Nephrology, Mexico City (1972).
[9] D. BEN-ISHAY, R. SALITERNICK and A. WELNER, Experientia *28*, 1321 (1972).
[10] J. R. SACHS and L. G. WELT, J. clin. Invest. *46*, 65 (1967).
[11] J. D. GARDNER, A. P. SIMOPOULOS, A. LAPEY and S. SHIBOLET, J. clin. Invest. *51*, 1565 (1972).