of FFA release is smaller in the brown fat of cold-acclimatized rats in the present study.

With regard to the mechanism in the lipolytic action of glucagon on brown adipose tissue, SKALA et al.[16] have described that the adenylcyclase receptor for glucagon is not found in the brown adipose tissue. Brown adipose tissue is richly innervated by 2 kinds of sympathetic nerves, the one to the vessels derived from the sympathetic chain, and the other to the parenchymal tissue derived from the intrinsic ganglia[17]. Thus, it is likely that the rise in brown fat venous FFA concentration due to glucagon is secondary to a rise of catecholamine level in the brown adipose tissue as well as the circulatory blood elicited by glucagon. However, it is unlikely, since the response to glucagon was unchanged after injection of beta-receptor blocker, propranolol, which is known to suppress lipolytic as well as calorigenic action of catecholamines[18] as seen in the table. Neither calorigenesis nor increase in blood flow by glucagon is blocked by propranolol[12]. Consequently, it seems reasonable to conclude that glucagon, like catecholamines, acts directly on the brown adipose tissue in its lipolytic and calorigenic actions. The increase in blood flow through the brown adipose tissue by glucagon infusion might be partly responsible for the increased release of FFA from this tissue, although it is likely that the increase in blood flow is secondary to the metabolic action of glucagon[12]. In this connection, it is interesting to refer to the recent report suggesting that cold acclimatization results in the decreased activity of phosphodiesterase in the brown adipose tissue of rats, inducing an increased lipolysis in this tissue[19]. Thus it would appear worthwhile to investigate whether glucagon could influence phosphodiesterase activity of brown adipose tissue.

It is now widely accepted that heat production in the brown adipose tissue is governed by the sympathetic nervous system[2]. However, HULL[20] has observed that beta-blocker, pronethalol, blocked the norepinephrine-induced calorigenesis in newborn rabbits, but it did not inhibit the calorigenic response to cold. The present result, together with that of Hull'sone, seems to suggest that glucagon may act synergistically with catecholamines in determining the level of brown adipose tissue metabolic process in cold acclimatization.

The brown adipose tissue has been shown to take up glucose actively from the circulation, especially in cold-acclimatized animals[21]. However, in the present study no significant difference was observed in the elevation of blood glucose concentration between the systemic venous and brown fat venous bloods in both warm-acclimatized and cold-acclimatized animals.

[16] J. SKALA, P. HAHN and T. BRAUN, Life Sci. *9*, 1201 (1970).
[17] D. M. DERRY, E. SCHOENBAUM and G. STEINER, Can. J. Physiol. Pharmac. *47*, 57 (1969).
[18] J. HIMMS-HAGEN, Pharmac. Rev. *19*, 367 (1967).
[19] R. BERTIN and R. PORTET, Can. J. Biochem. *53*, 1301 (1975).
[20] D. HULL, J. Physiol. *173*, 13 (1964).
[21] G. STEINER and G. F. CAHILL, jr., Am. J. Physiol. *207*, 840 (1964).

Arousal-induced increase of cortical [K^+] in unrestrained rats

H. Korytová*

Institute of Physiology, Czechoslovak Academy of Sciences, Budějovická 1083, 142 20 Praha 4 – Krč (Czechoslovakia), 5 August 1976

Summary. Changes of extracellular concentration of brain potassium $[K^+]_e$ were studied in lightly anesthetized unrestrained rats with ion-selective K^+-microelectrodes introduced into the cerebral cortex with a head-mounted microdrive system. Nociceptive stimuli elicited EEG arousal lasting for 47 sec on the average which was accompanied by an increase of $[K^+]_e$ from 3.0 mM to 3.31 $\pm$ 0.04 mM.

The $[K^+]$-sensitive microelectrodes[1] made it possible to examine the role of K^+-ions in various normal and pathological brain functions. The level of $[K^+]_e$ in the cerebral tissue was found to be equal to the potassium concentration in the CSF (3 mM), under resting conditions[2], and to increase to 60–80 mM during spreading depression or anoxic depolarisation[3–5] and to about 10 mM during epileptic activity[4, 6–8]. Lower $[K^+]_e$-increments were observed in the spinal cord and higher brain centres after electrical stimulation of peripheral nerves or after application of adequate sensory stimuli[9–11].

All the above studies were made in deeply anesthetized (and often curarized) animals, rigidly fixed in the head holder of the stereotaxic apparatus. Such experimental conditions preclude the use of K^+-selective electrodes for examination of $[K^+]_e$-shifts accompanying various behavioral states. The aim of the present paper was to modify the K^+-electrode technique to make it suitable for measurements in unrestrained animals by reducing the movement artefacts due to high impedance of the electrode and to changes of input-ground capacity and electrostatic induction.

K^+-microelectrodes were prepared according to the technique described by Walker[1] and modified by Vyskočil and Kříž[12]. Glass micropipettes (1.65 mm external diameter, 20 mm long, with a 5 mm long shank) contained a 0.5 M KCl solution contacting a 200 μm high column of the liquid ion exchanger (Corning Code 477317) in the siliconized tip. Their resistance was around 10^8 Ω. In order to simplify the electrode system a micropipette of the same shape but filled with 0.9% NaCl was used for field potential recording from the same region. Efficient grounding of the animal was provided by a low resistance Ag-AgCl-electrode (4 mm^2) contacting the cortical surface adjacent to the point of microelectrode insertion.

Adult hooded rats were anesthetized with urethane (0.9 g/kg) and a trephine opening 5 mm in diameter was made over the occipital cortex. The microdrive system and its fixation to the skull is shown in figure 1. 3 anchoring bolts placed around the trephine hole were used for fixation of a 3 mm high plastic ring (11) with internal diameter of 4 mm, held by 3 supporting rods (12) about 3 mm above the exposed dura (13) and bone (8). 2 silver screws (2 mm) fixed in the skull over the frontal cortex

served for EEG recording. Vertical microdrive consisted of a teflon cylinder (6) fitting into the implanted ring. Parallel to the axis of the cylinder were two 2-mm-channels. One of them served as a receptacle for a silver rod (9), the lower end of which was electrolytically covered with a layer of AgCl. The other channel was used as a guiding tube for the K^+-microelectrode (10). The latter was clamped in an electrode holder (4) which could be moved up and down by a screw (3) rotating in bearings carried by a metal frame (2), fixed to the teflon cylinder (7). The frame also carried a FET signal follower (1), the gate of which was connected to a short Ag-AgCl-electrode (5) contacting the fluid in the microelectrode tube.

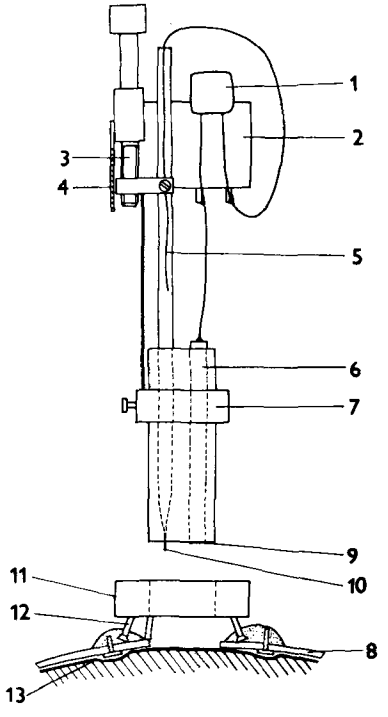

Fig. 1. Scheme of the microdrive system used for $[K^+]_e$-recording in the cerebral cortex of unrestrained rat. For decription see the text.

Calibration of the electrode system was made in various concentrations of KCl (4 mM and 40 mM) with added 150 mM NaCl. First the Ag-AgCl-electrode-NaCl capillary electrode pair was immersed into the test solutions and the respective DC potentials were recorded. The same procedure was repeated with the Ag-AgCl-electrode-K^+-electrode pair. The K^+-potential was computed as the difference between the above values. Low bias current of the FET probe (less than 10^{-11} A) and low electrode impedance (less than 10^8 Ω) prevented distortion of results by excessive input offset potentials.

The electrode was then inserted 1 mm below the cortical surface and the recording was started. After conclusion of the recording the K^+-electrode was calibrated again and replaced with the NaCl filled capillary. The recording was repeated under similar conditions with the NaCl-electrode introduced into the same position. Subtraction of the latter field potential changes from the former K^+-electrode potential, representing a sum of the potassium potential E_K and of the field potential, made it possible to isolate E_K and to estimate the corresponding $[K^+]_e$-shifts.

The $[K^+]_e$-values established by the above procedure in rats under light urethane anesthesia were similar to those found in earlier studies using simultaneous insertion of the K^+-electrode and NaCl reference electrode to the same area. In spite of small movements of the animal, the recording was relatively free of artefacts both during rest and during arousal induced by nociceptive stimuli (pinching of the tail). Figure 2 shows a typical example of arousal reactions induced in the same rat by such stimuli. EEG was recorded between the silver screws in the frontal and parietal bones (2–3). Whereas the NaCl-electrode recorded only negligible slow potential changes during EEG arousal (figure 2, above) the K^+-electrode showed marked positive shifts reaching an amplitude of 1 to 1.5 mV (figure 2, below).

Slow potential changes accompanying comparable arousal reactions (average duration 47 $\pm$ 5 sec) were recorded in 5 rats with the NaCl-electrode (n = 52) and with the K^+-electrode (n = 57). EEG desynchronisation was accompanied by a low negativity of the NaCl-filled electrode which did not exceed 100 μV on the average. The K^+-electrode recorded under the same conditions a much higher potential change averaging 1.1 $\pm$ 0.15 mV. Taking

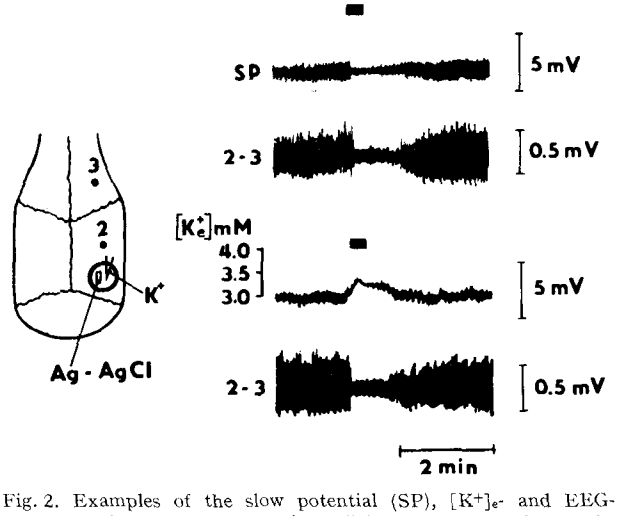

Fig. 2. Examples of the slow potential (SP), $[K^+]_e$- and EEG-recordings during arousal reactions elicited by compression of the tail. The scheme on the left side shows the location of electrodes. The slow potential and $[K^+]_e$ are recorded between the Ag-AgCl-electrode and the intracortical (1 mm deep) capillary electrode. The horizontal rectangle indicates the duration of the nociceptive stimulus. Calibration: 5 mV for SP and E_K, 0.5 mV for the EEG. The $[K^+]_e$-scale is shown on the left side of the E_K-record.

* Acknowledgment. I wish to express my sincere gratitude to Dr. Bureš from the Institute of Physiology, Czechoslovak Academy of Sciences, for his kind help in the preparation of this article.

1 J. L. Walker, Analyt. Chem. *43*, 89 A (1971).
2 A. Van Harreveld, Butterworths, Washington, D. C. (1966).
3 F. Vyskočil, N. Kříž and J. Bureš, Brain Res. *39*, 255 (1972).
4 D. A. Prince, H. D. Lux and E. Neher, Brain Res. *50*, 489 (1973).
5 K. J. Futamachi, R. Mutani and D. A. Prince, Brain Res. *75*, 5 (1974).
6 H. G. Lux, E. Neher and D. A. Prince, Pflügers Arch., suppl. 332, R 89 (1972).
7 J. Bureš, I. von Schwarzenfeld and G. Brožek, Epilepsia *16*, 111 (1975).
8 J. Machek, E. Ujec and V. Pavlík, Physiol. Bohemoslov. *24*, 41 (1975).
9 L. Vyklický, E. Syková, N. Kříž and E. Ujec, Brain Res. *45*, 608 (1972).
10 N. Kříž, E. Syková, E. Ujec and L. Vyklický, J. Physiol. *238*, 1 (1974).
11 K. Krnjević and M. E. Morris, Can. J. Physiol. *53*, 912 (1975).
12 F. Vyskočil and N. Kříž, Pflügers Arch. *337*, 265 (1972).

into account that the K^+-electrode potential was practically uncontaminated by the negligible field potential, the above value could be interpreted as an E_K-potential corresponding to a $[K^+]_e$-increase from 3.0 mM to 3.31 $\pm$ 0.04 mM. The average duration of the $[K^+]_e$-increase was 37 sec and was correlated with the duration of EEG desynchronisation. The maximum $[K^+]_e$ was attained 13 $\pm$ 2 sec after desynchronisation onset.

The above results confirm the feasibility of $[K^+]_e$-recording in unrestrained rats. Although in the present study the movement was limited by light anesthesia and recording in freely moving animals was severely disturbed by artefacts, it is conceivable that further improvement of the technique may produce supression of capacitative and electrostatic interference and make it possible to record $[K^+]_e$-changes in behaving animals.

The amplitude of E_K is several times higher than the corresponding field potential changes[13]. The $[K^+]_e$-changes seem to be much more sensitive and straightforward index of sustained activity of large neuronal populations than the corresponding slow potential changes. It can be expected that K^+-electrodes will provide an important method in tracing neural circuits participating in various behavioral processes.

13 H. Caspers, Proc. int. Union Physiol. Sci, XXII Int. Congress, Leiden, vol. I, part I, p. 442 (1962).

Effects of hypergravity on rat fertility, pregnancy, parturition and survival

J. Ishay and L. Barr-Nea

Department of Physiology and Pharmacology and Department of Histology and Cell Biology, Sackler School of Medicine, Tel Aviv University, Ramat Aviv (Israel), 2 June 1976

Summary. Persistent centrifugation (at 1.18 $\times$g to 1.47 $\times$g) of pregnant rats reduced the number of deliveries and the survival time of the newborns. The extent of the damage increased with increasing distance from the axis. Male fertility was reduced.

Introduction. In previous papers various pathological changes have been reported to occur in animals kept under persistent centrifugation[1-6]. In the present study we describe the effects of centrifugation on fertility, pregnancy, delivery and survival of rats under persistent centrifugation at relatively low additional g-forces.

Materials and methods. A special four-armed centrifuge was designed and constructed to accomodate the regular rat breeding cages (BC). 5 such cages were attached firmly to each arm at different distances from the center of rotation. The resultant forces which acted upon the cages when the centrifuge rotated at 25 rpm, were as follows: first cage at 86 cm from the center – 1.27 $\times$g, second cage at 104.5 cm from the center – 1.34 $\times$g, third cage at 125.0 cm – 1.41 $\times$g, fourth cage at 141.5 cm – 1.43 $\times$g, and fifth (terminal) cage at 150.0 cm – 1.47 $\times$g. Concommitantly 2 controls were used: a) a rotating control of 2 BC's placed in opposition at a distance of only 20 cm of the center of the centrifuge (resultant g-force 1.04); b) a stationary control of 6 BC's placed on the floor behind the centrifuge (figure 1).

Once daily, but at different times[7], the centrifuge was stopped for 15 min in order to check the animals, supply them food and water, clean the cages and service the machine.

Test design. The centrifuge was activated within a special room, under fluorescent illumination and at constant temperature of 26 °C. Each animal was spun for 4 weeks,

Effects of increasing gravitational force on the reproduction pattern of adult rats and survival of the offspring

Cage No.	g	Females placed in centrifuge at mid-pregnancy (day 10–13)			Females placed in centrifuge at the beginning of pregnancy (2–4 days after confirmed mating)		
	Resultant forces	Number of normal deliveries	Number of*** live newborns (per litter)	Live span of newborns in days*** (per litter)	Number of normal deliveries	Number of*** live newborns (per litter)	Life span of newborns in days
Center (rotating control)	1.04	5	8, 8, 11, 9, 8 (9)	normal (more than 60 days)	5	10, 11, 7, 8, 9 (9)	normal
1	1.27	5	8, 8, 9, 7, 10 (8)	normal	5	9, 10, 7, 8, 8 (8)	normal
2	1.34	5	10, 7, 9, 7, 8 (8)	normal	5	8/9, 9, 7, 6, 5/6 (7)	normal
3	1.41	5	8, 11/12****, 8/9, 10, 7/9 (9)	4 groups normal last litter = 1 day	5	6/8, 5/8, 7, 8/9, 7 (6.5)	normal
4	1.43	4/5*	6/8, 8, 8/9, 8/11 (7.5)	4, 3, 6, 10 (6)	3/5	8/9, 5/7, 4/7 (6)	normal
5	1.47	3/5**	7/12, 3/7, 5/7 (5)	2, 1, 2 (2)	1/5	4	3.5 days

* 1 abortion, ** abortions, *** mean in brackets, **** ratio of live newborns/total litter. The delivery rate and life span of rats in stationary control BC's was normal for that breed.