Postnatal variations of extracellular free calcium levels in the rat. Influence of undernutrition

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Summary. Ontogenetic changes in calcium activity were directly measured using an ion-selective micropipette in rat blood plasma and olfactory bulb extracellular fluid. Significant differences were observed according to the age and the nutritional state of the animal.

Calcium ion is known to play a number of important roles in the functioning of the nervous system: control of neuronal excitability by direct effect on the Na^+ permeability in cell membranes[2], and regulation of neurotransmitter synthesis and release[3,4]. Modulation of ionized calcium concentration in the extracellular microenvironment is demonstrated to be of the greatest physiological importance[5]. This study is part of an electrophysiological and biochemical investigation of the effects of age and nutrition on rat olfactory bulb ontogenesis, particularly on mitral cell activity[6]. The concentration of total calcium in the blood of newborn rats was observed to decrease markedly during the 1st postnatal hours[7]; this decrease was followed by a progressive increase between 12 and 36 h after birth[8]. On the other hand, ionized calcium concentrations in blood and brain extracellular fluid were not estimated by these authors during postnatal development. These determinations are the aim of the present work.

Material and methods. Experiments are conducted on Wistar rats of our inbred laboratory strain. The offspring are investigated from birth (day 1) to 60 days of age (adult). This immediate postnatal period, characterized by an elevated anabolic capacity, is one of the most vulnerable phases in the life of the rat, at least in terms of its response to dietary modifications. The undernutrition of neonatal rats has been widely demonstrated to slow the ontogenetic evolution in the brain; therefore this evolution is compared in 2 groups of animals. In the control group, the young are suckled in litters of 6 and the mother fed ad libitum with an optimum standard laboratory diet. The undernourished group consists of the progeny of dams (12–15 animals/litter), fed ad libitum, from delivery and during 3 weeks, with a low protein (10% casein) isocaloric purified diet; the milk produced by the malnourished dam is demonstrated not to differ significantly in quality from that of well-nourished dams, but is reduced in volume[9,10]. Thus the preweanling offspring of protein malnourished dams are nutritionally deprived with respect to both protein and total calories[11]; a 76% reduction in body weight gain and a 28% reduction in nervous structure wet weight gain are then observed. Undernourished young rats, after weaning, (21 days) have free access to the control diet until 60 days of age; during this nutritional rehabilitation period the physical characteristics of the underfed rats improve noticeably. Rats are injected with Flaxedil and placed under respiratory assistance. 6–23 animals are tested at each age. The determinations of Ca^{++} activity in olfactory bulb extracellular fluid and blood plasma are performed with the fast responding ion selective microelectrode as described by Oehme et al.[12]. Micropipettes are drawn from thetacapillaries; tip diameters of the electrode are 2–3 µm. 1 channel is filled with 0.1 M $CaCl_2$, the other with 0.1 M KCl. The inner wall of the ion-sensitive side is made hydrophobic at its tip by a 5% dichloro-dimethyl-silane solution introduced by successive air pressure-vacuum cycles. The Simon neutral carrier ion exchanger is then introduced up to a height of 200 µm by applying vacuum on the calcium channel. Chlorinated silver wires are then introduced in both channels. The Ca^{++} sensitive electrodes with an impedance of 2.10^9 Ω, are connected to a Grass HIP-16 microelectrode preamplifier having an input impedance of 10^{11} Ω. The amplifier output is connected to a storage oscilloscope DM 64 Telequipment. Ca^{++} electrodes are calibrated with $CaCl_2$ solutions; calibrating solutions contained 140 mM NaCl to simulate the ionic strength of the extracellular environment; they respond with an average of 30.1 ± 0.32 mV to a 10-fold change in Ca^{++} concentration. The selectivities relative to Mg^{++} and Na^+ are verified to be high. Blood samples are drawn by cardiac puncture and collected in heparinized tubes; measurements are carried out immediately but no significant changes in calcium activity are noted when samples are stored for 24 h at +4 °C. In the case of extracellular fluid analysis, a 1.0 mm in diameter hole is drilled through the skull over the olfactory bulb. The electrode tip is sunk to the mitral cell layer; the immediate vicinity of mitral cells is demonstrated by a simultaneous recording of extracellular spikes[6].

Results and discussion. 1. Blood plasma levels (figure 1). In newborn animals, calcium activity in the plasma is 2.44 ± 0.13 mM/l. A 28% decrease is observed within the following hours; this decrease is followed, at day 3, by an increase up to 2.10 ± 0.12 mM/l. A parallel evolution in total calcium is noted[8]. But from day 7, while total calcium gradually increases[13], calcium activity progressively decreases up to 1.48 ± 0.05 mM/l at 35 days of age. Slightly lower levels are observed in adult animals. Thus the

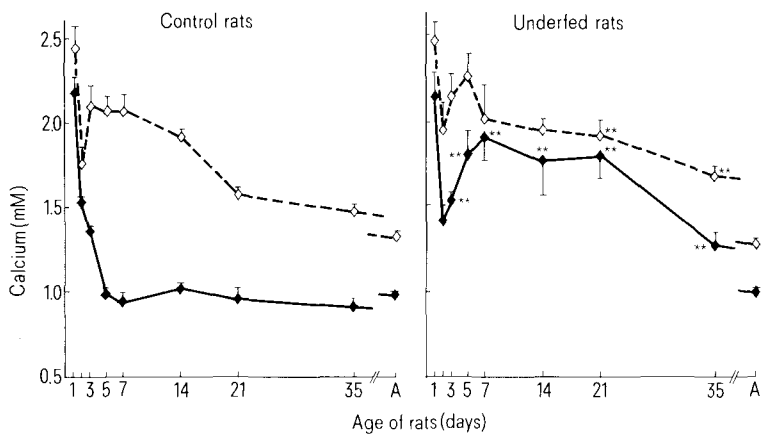

Fig. 1. Developmental changes of calcium activity in rats from birth to 60 days of age. Postnatal evolution in control (left panel) and underfed (right panel) rats: calcium activity levels in blood plasma (open symbols, broken line) and in olfactory bulb extracellular fluid (black symbols, continuous line) are indicated. The results are expressed as means ± SEM. The differences between control and underfed groups are assessed by the t-test. *$p < 0.01$; **$p > 0.005$ (right panel).

percentage of ionized calcium in plasma decreases from about 80% at birth to 60% at weaning and 50% in older rats. No statistically significant differences are observed between control and underfed rats from birth to 14 days of age. But no decrease in calcium activity is observed in underfed animals during the third postnatal week and thus, at 21 days, activity is significantly higher (22%). This difference gradually disappears during the rehabilitation period.

2. Olfactory bulb extracellular fluid levels (figure 1). Concentrations of ionized calcium in olfactory bulb extracellular fluid are lower than in blood plasma; the postnatal evolution is strikingly different in control and underfed rats.

A. Control group: In newborn animals, calcium activity in the vicinity of mitral cells is 2.18 ± 0.09 mM/l; it decreases abruptly up to day 5. The unstimulated base line values are then typically maintained between 0.92 ± 0.05 and 1.02 ± 0.04 mM/l; similar results are reported in adult rat cerebellum[5] and in cat somato-sensory cortex[14]. These data demonstrate a decrease in extracellular fluid/blood plasma ratio of calcium activities with age: within the first 2 postnatal days the ratio value is 0.89 and 0.87; a decrease to 0.65 at day 3 and constant values of about 0.50 are observed from day 5 to day 14. A gradual increase up to 0.74 is then noted. Significant variations in calcium activity are evoked by repetitive stimulation (4 sec train of 100 Hz electrical stimulation) of the contralateral olfactory bulb; these changes vary in amplitude and duration according to the developmental stage of the animal. In 21-day-old control rat (figure 2) extracellular calcium level falls to about 0.87 mM/l (94% of the base line concentration); the minimum is reached within 6 sec. This fall in a_{Ca} may result either from increased influx of calcium into the intracellular space (neuronal and/or glial)[5,14] probably by facilitated diffusion[15] or from binding at cell membrane[16]. Following stimulus cessation, a slight 'peak' in extracellular a_{Ca} (0.06 mM in amplitude, 2.1 sec in duration) is observed; this peak develops during ontogenesis, it supports the idea that active extrusion mechanisms (presumably a specific calcium-ATPase system[17] or a sodium-calcium exchange mechanism[18,19]) do appear in the olfactory bulb. Subsequent to this 'peak' a_{Ca} decreases to 0.75 mM/l and then progressively returns to base line levels; this secondary decrease persists for about 19 sec.

B. Underfed group: Following the initial decrease from 2.16 ± 0.14 to 1.42 ± 0.09 mM/l within the first 2 postnatal days, similar to that described in the control group, calcium activity in the extracellular fluid of underfed rats progressively increases up to 1.92 ± 0.13 mM/l at day 7 (figure 1). Unstimulated base line values of 1.8–1.9 mM/l, significantly higher than those noted in control rats, are then maintained over the underfeeding period. Extracellular fluid/blood plasma ratio of calcium activity is held at a mean value of 0.93. Nutritional rehabilitation period is characterized by a progressive drop in calcium activity to 1.00 ± 0.03 at 60 days of age, ratio value attaining 0.77; these values are similar to those observed in control group at the same age. At 21 days of age, repetitive electrical stimulation induces a fall in calcium level, the amplitude of which is 40% smaller than observed in control group; no intermediary peak could be noted (figure 2). Calcium metabolism is thus seriously disturbed in underfed rats. Differences observed between control and underfed animals could explain the delay in ontogenesis of inhibitory responses as described elsewhere[6].

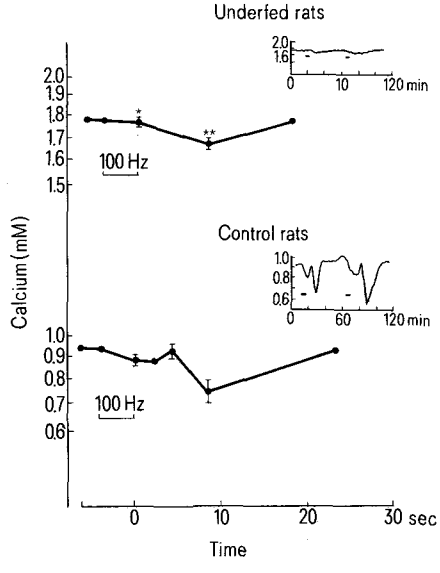

Fig. 2. Calcium activity changes in olfactory bulb extracellular fluid in response to contralateral olfactory bulb stimulation at 100 Hz in 21-day-old rats. Upper level: underfed rats; each point represents the mean±SEM of 13 determinations. A direct recording of a typical experiment, with 2 successive electrical stimulations, is shown in the inset. Lower level: control rats; each point is the mean±SEM of 15 determinations. The inset is a direct recording of the response to 2 successive stimulations. The differences in amplitude of the calcium activity changes between control and underfed groups are assessed by the t-test.

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