Introduction

The proper aim of the presented paper is a comparison of different manners of blood sampling when basal glycemia and/or glucose tolerance curve is monitored. The basic problem is represented by restraining of the stress reaction which can take part in the procedure of blood sampling.

Kelsey (2) and Mangili et al. (5) when studying the basal ACTH secretion obtained all basal samples under the ether anaesthesia but in less than 90s before a stress-induced increase in ACTH secretion could occurred. Sampling was performed by cardiac puncture.

Placing the animal for 3 minutes in an ether vapor saturated area (4) is frequently used as a systemic stressor. On the other hand, Kelsay (2) and Mangili et al (5) used ether narcosis when basal ACTH was monitored. Thus it is apparent that ether must be taken into consideration as stressor only under some condition, i.e., when there is sufficient time delay between the beginning of ether vapor inhalation and the blood sampling used for determination of ACTH and/or plasma corticosterone.

In our recent paper we are comparing basal glycemia when three types of blood sampling were used: two of them were realized under narcosis, i.e., under pentobarbital and/or ether narcosis, and the last one when sampling was realized by decapitation without narcosis.

Methods

Animals

Experiments under the ether narcosis were performed in the rats of Wistar strain, in the genetically hypertensive obese rats (SHR/N-cp obese) of Koletsky type (3) and in their lean sibling (SHR/N-cp lean). Lean SHR/N-cp rats represent dominant non-obese homozygotes and heterozygotes whereas their obese siblings are recessive homozygotes (cp-cp). The abnormal animals were obtained by Koletsky (3 when mating spontaneously hypertensive rat (Okamoto-Aoki strain) with a normotensive Sprague-Dawley male rat. The genetically obese animals appeared after several generations of selective inbreeding of hypertensive off-springs of the original cross.

After weaning at the age of 30 days the animals were kept in groups of four and supplied with water and ST pelleted diet ad libitum. Monitoring was realized in adult rats.

Blood glucose sampling

Blood was sampled to heparinized capillaries (from retrobulbar plexus under light ether anaesthesia) and after 121
about one week delay the second sampling was realized when animals were decapitated without narcosis. Thus, the animal represents the control for itself.

Under the term of light ether anaesthesia we understand the procedure which enable us the blood sampling before the development of stress induced changes in glycemia. The ether narcosis is applied in glass vessel (10x10x20 cm) where at the bottom is metal grid which is covered by paper-wool. The grid and paper-wool make impossible the direct contact of the ether liquid with the animal. For every narcotization cca 3 ml of ether was applied. The glass vessel with the cover by a lid and the behaviour of the animal is observed. When the rat falls asleep (50-60 s after beginning of narcotization), the blood sampling from retrobulbar plexus is performed and finished up to 60-70 s after the beginning of narcotization.

**Basal glycemia and glucose tolerance Blood was sampled to heparinized capillaries (from retrobulbar plexus under light ether anaesthesia) before glucose loading (basal glycemia) as well as 30, 60, 120 and 180 min after glucose loading (area under the glucose tolerance curve).** Basal glycemia and glucose tolerance was monitored under ether and Nembutal anaesthesia (50 mg/kg, i.p.) as well. Basal glycemia without narcosis was obtained when blood sample was taken by decapitation.

**Successive blood sampling without glucose loading**

To verify the possible effect of repeated sampling two different experiments were performed. In the first case we used identical arrangement which we used in glucose tolerance test, i.e., sampling was realized at time 0, 30, 60, 120 and 180 min in two groups of animals, i.e., after 14 hour starvation and in group without starvation.

The last series of experiments were performed only under the ether anaesthesia when two minutes intervals between individual sampling steps were done in the animals after 14 h starvation and in group without starvation. Measurements were carried out in males of Wistar strain.

**Results**

When compared basal glycemia obtained under light ether anaesthesia and under Nembutal anaesthesia (sampling in both cases from retrobulbar plexus - see Methods). statistically significant differences were obtained only in one group of rats, i.e., in males (Table 1).

The lower level was obtained under decapitation without narcosis. In the mentioned comparison the animal represents the control for itself.

When compared strain differences in the basal glycemia (Table 1) the lean Kolcetsky rats show increase in relation to Wistar rats, except glycemia in females where elevation of basal glycemia attained statistical significance only under the sampling by decapitation without narcosis. Considering the strain differences in basal glycemia statistical significance was attained only in males when sampling by decapitation without narcosis was used (Table 1).

| Group | Control | Decapitation | Ether | Ether + Nembutal |
|-------|---------|--------------|-------|-----------------|
| NR M  | 3.51±0.81 | 3.26±0.24 | 3.17±0.22 | 3.17±0.22 |
| NR F  | 3.62±0.26 | 3.71±0.26 | 3.71±0.26 | 3.71±0.26 |
| SHR M | 4.67±0.43 | 4.71±0.43 | 4.71±0.43 | 4.71±0.43 |
| SHR F | 4.10±0.20 | 4.10±0.20 | 4.10±0.20 | 4.10±0.20 |
| SHIR M| 4.13±0.17 | 4.39±0.20 | 4.39±0.20 | 4.39±0.20 |
| SHIR F| 4.54±0.46 | 4.86±0.86 | 4.86±0.86 | 4.86±0.86 |

Means±SD. Statistical significance between basal glycemia when sampling was carried out from retrobulbar plexus under the light ether anaesthesia and/or by decapitation without narcosis was evaluated by parametric paired t-test and controlled by non-parametric Wilcoxon test for matched pairs. Intergroup differences were evaluated by unpaired parametric test and controlled by non-parametric Mann-Whitney two sample (non-matched) test. Abbreviations: SHR - lean Kolcetsky rats, SHIR - obese Kolcetsky rats; M - males; F - females; n - number of rats in group. D - significance of differences between the lean Kolcetsky rats and the rats of Wistar strain; d - significance of differences between obese and lean Kolcetsky rats; D and d/pp<0.01. The measure of statistical significance in all cases according to the results in non-parametric test.

Comparing the basal glycemia under the conditions of repeated sampling (Table 2) of blood at 30, 60, 120 and 180 min intervals (i.e., in the time schedule when we used in the monitoring glucose tolerance), we have not found any differences under the conditions of Nembutal and/or ether anaesthesia. In both cases the blood was sampled to heparinized capillaries from retrobulbar plexus. When we compare the glycemia under the mentioned time schedule in the starved and/or unstarved animals, the elevation shows statistical significance with two intervals in the rats without starvation.

| Glycemia | Control | Ether | Ether + Nembutal |
|---------|---------|-------|-----------------|
| 24      | 4.29±0.71 | 4.29±0.71 | 4.29±0.71 |
| 48      | 4.71±0.43 | 4.71±0.43 | 4.71±0.43 |
| 72      | 4.10±0.20 | 4.10±0.20 | 4.10±0.20 |
| 120     | 4.13±0.17 | 4.39±0.20 | 4.39±0.20 |
| 180     | 4.54±0.46 | 4.86±0.86 | 4.86±0.86 |

The factor of starvation is profoundly expressed also under the repeated sampling of blood at two min intervals when the repeated ether anaesthesia was applied (Table 1). There is profound difference not only in the level of basal glycemia (see point 1), but quite different steepness is apparent, being much higher in the group without starvation. When the glycemia at the first sec is taken as 100%, then the glycemia at 16th sec represents 153% (see upper row in Table 3, i.e., under the starvation) and the glycemia at 15th sec (see lower row in Table 3, i.e., under the free access to pellet) represents 193%.

**Table 3: Means SSD.**

| Group | Control | Ether | Ether + Nembutal |
|-------|---------|-------|-----------------|
| 1     | 4.24±0.49 | 4.29±0.71 | 4.29±0.71 |
| 2     | 4.29±0.71 | 4.29±0.71 | 4.29±0.71 |
| 4     | 4.71±0.43 | 4.71±0.43 | 4.71±0.43 |
| 8     | 4.10±0.20 | 4.10±0.20 | 4.10±0.20 |
| 12    | 4.13±0.17 | 4.39±0.20 | 4.39±0.20 |
| 18    | 4.54±0.46 | 4.86±0.86 | 4.86±0.86 |

The top row: animals under the 14th starvation (n=4), the bottom row: animals without starvation with pellet diet and light ether anaesthesia (n=4). The level of glycemia at individual stages of experiments (t vs t-minus min, 2nd min versus 3rd min, 4th min versus 5th min etc).

Differences were statistically significant only in males when sampling was performed under ether and/or Nembutal anaesthesia (Table 2, the upper two rows). As to the pentobarbital anaesthesia (see in our experiments Nembutal), Moss (6) found that this type of narcotic did not alter any ACTH levels.

On the other hand, when the intersampling intervals are 2 sec., then there can be observed profound reaction of the gastation of stress reaction in the form of elevated glycemia (see Table 3). It is apparent that the augmentation of stress reaction is highly state dependent. i.e., the augmentation is twofold higher in the animals which are restricted to starvation. Where is a proper reason of the mentioned differences it is not clear. It cannot be a priori excluded that the different state of available oxygen depots can be judged as a main intervening variable.

This paper was supported By Internal Grant Agency of Ministry of Health of the Czech Republic No 36843. The authors wish to thank Carl T. Hansen, Animal Genetics Division, National Institute of Health, Bethesda, USA, for providing the genetically hypertensive rats of Kolcetsky type.

**References**

1. Hefo VP. The role of the medial hypothalamic nervous afferents in inducing hyperglycaemia in rats by certain stressors. Rev Roum Endocrin 1972;9:97-102.
2. Kelsay JE. Role of pituitary-adrenocortical system in mediating avoidance behavior of rats with septal lesions. J Neurosurg Psychol 1982;45:278-280.
3. Kolcetsky S. Pathologic findings and laboratory data in a new strain of hypertensive rats. Am J Pathol 1975;80:3119-40.
4. Kvetnianskis R, Dobrakova M, Ježová D, Opalíová Z, Lichardus B, Makara G. Hypothalamic regulation of plasma catecholamine levels during stress; effect of vasopressin and CRF. In: Stress - Neurochemical and Humoral Mechanisms. GR van Loon, R.Kvetnianskis, R.McCarty, J Axelrod (Eds), New York:Gordon and Breach Science Publishers, 1989:549-70.
5. Mangili G, Motta M, Martini L. Control of adrenocorticotropin hormone secretion. Cit. Kelsey JE J Comp Physiol Psychol 2001;137:1860-1864.
6. Moss IR, Inman JG, Porter JC, Faucher DJ. Ontogeny of plasma, CSF and brainstem ACTH in piglets: effects of hypoxia and anaesthesia. Neuroendocrinology 1990;51:586-91.

Submitted April 1998. Accepted May 1998.

**Doc. MUDr. PhDr. Vítĕral Šolca, CSc., Institute of Experimental Neurosurgery, Charles University Faculty of Medicine and Teaching Hospital, 500 05 Hradec Králové, Czech Republic.**
about one week delay the second sampling was realized when animals were decapitated without narcosis. Thus, the animal represents the control for itself.

Under the term of light ether anaesthesia we understand the procedure which enable us the blood sampling before the development of stress induced changes in glycemia. The ether narcosis is applied in glass vessel (10x10x20 cm) where at the bottom is metal grid which is covered by paper-wool. The grid and paper-wool make impossible the direct contact of the animal with liquid ether. For every narcotization cca 3 ml of ether was applied. The glass vessel with the cover by a lid and the behaviour of the animal is observed. When the rat falls asleep (50 - 60 s after beginning of narcotization), the blood sampling from retrobulbar plexus is performed and finished up to 60 - 70 s after the beginning of narcotization.

**Basilica glycemia and glucose tolerance Blood was sampled to heparinized capillaries (from retrobulbar plexus under light ether anaesthesia) before glucose loading (basal glycemia) as well as 30, 60, 120 and 180 min after glucose loading (area under the glycemia tolerance curve).** Basal glycemia and glucose tolerance was monitored under light ether anaesthesia (50 mg/kg, i.p.) as well. Basilica glycemia without narcosis was obtained when blood sample was taken by decapitation.

**Successive blood sampling without glucose loading** To verify the possible effect of repeated sampling two different methods were performed. In the first one we used identical arrangement which we used in glucose tolerance test, i.e., sampling was realized at time 0, 30, 60, 120 and 180 min after glucose loading (area under the glycemia tolerance curve). Basal glycemia and glucose tolerance was monitored under light ether anaesthesia (50 mg/kg, i.p.) as well. Basilica glycemia without narcosis was obtained when blood sample was taken by decapitation.

When compared basal glycemia obtained under light ether anaesthesia and under Nembutal anaesthesia (sampling in both cases from retrobulbar plexus - see Methods). statistically significant differences were obtained only in one group of rats, i.e., in SHR group (Table 1). The lower level was obtained under decapitation without narcosis. In the mentioned comparison the animal represents the control for itself.

When compared strain differences in the basal glycemia (Table 1) the lean Koloteca rats show increase in relation to Wistar rats, except glycemia in females where elevation of basal glycemia attained statistical significance only under the sampling by decapitation without narcosis. Considering the substations differences in basal glycemia statistical significance was attained only in males when sampling by decapitation without narcosis was used (Table 1).

| Group | n | retrobulbar plexus (mmol/l) | decapitation (mmol/l) | P |
|-------|---|-----------------------------|-----------------------|---|
| SHR M | 8 | 3.62±0.64 | — | 0.18 |
| SHR F | 8 | 3.62±0.64 | — | 0.18 |
| SHR M | 8 | 4.67±0.65 | — | 0.17 |
| SHR F | 8 | 4.67±0.65 | — | 0.17 |

Table 1: Basal glycemia under ether and Nembutal anaesthesia

The factor of starvation is profoundly expressed also under the repeated sampling of blood at two min intervals when the repeat ether anaesthesia was applied (Table 1). There is profound difference not only in the level of basal glycemia (see point 1), but quite different steepness is apparent, being much higher in the group without starvation. When the glycemia at the first sec is taken as 100%, then the glycemia at 16th sec represents 153% (see upper row in Table 3, i.e., under the starvation) and the glycemia at 15th sec (see low row in Table 3, i.e., under the free access to pellet) represents 193%.

| Interval in min | 1 | 2 | 4 | 6 | 8 | 10 | 12 | 18 |
|----------------|---|---|---|---|---|---|---|----|
| Glycemia (mmol/l) | 4.24 | 4.29 | 5.01 | 4.73 | 5.25 | 5.73 | 5.78 | 6.55 |
| 10 | 5.62 | 4.66 | 4.93 | 5.15 | 7.95 | 8.37 | 8.79 | 9.67 |
| 20 | 8.25 | 10.47 | 10.55 | 10.20 | 12.29 | 12.04 | 13.61 | 15.27 |

Table 3: Means SSD. The top row: animals under the 14h starvation (n=4), the bottom row: animals without starvation with pellet diet and light ether anaesthsia (n=4) (The level of glycemia at individual experiments (t test versus min 1,2,3,4 min, 4th min versus 5th min etc.).

**Discussion**

It is apparent that basal glycemia obtained by sampling under the ether anaesthesia and the sampling by decapitation without narcosis is comparable. Moreover, our data obtained in normotensive rats of Wistar strain are comparable with the results presented by Heofoo (1). This author studied the basal glycemia in the males of Wistar as well. He removed the blood samples from tail veins. No more details are given in the paper mentioned above. He described three experiments with the following basal glycemia levels: 4.18±0.22 (n=7), 3.81±0.17 (n=10), and 3.80±0.16 (n=7). The data suggest that intergroup differences can represented about 0.2 mol/l. About the repeated narcotization with ether. Our data suggest that repeated application of ether shows the stress effect (in our case the elevation of glycemia) in dependance on the frequency of an exposition the animal to ether vapor. When the intersampling intervals are 30 sec or more, we can see that the level of glycemia is equal or near to basal glycemia (when sampling was made under the condition of starvation) or to the first sample (when sampling was made in the animal with free access to pellets (see Table 2). No augmentation in stress reaction can be found. No differences were found when sampling was performed under ether and/or Nembutal anaesthesia (Table 2, the upper two rows. As to the pentobarbital anaesthesia (see in our experiments Nembutal), Moss (6) found that this type of narcotic did not alter any ACTH levels.

On the other hand, when the intersampling intervals are 2 sec, then there can be observed modified in the elaboration of stress reaction in the form of elevated glycemia (see Table 3). But is apparent that the augmentation of stress reaction is highly state dependent, i.e., the augmentation is twofold higher in the animals which was exposed to the starvation. Where is a proper reason of the mentioned differences it is not clear. It cannot be a priori excluded that the different state of available glycogen deposits can be judged as a main intervening variable.

**References**

1. Heofoo VP. The role of the medial hypothalamic nervous afferents in inducing hyperglycaemia in rats by certain stressors. Rev Roum Endocrin 1972:97-102.

2. Kelsay JE. Role of pituitary-adrenocortical system in mediating avoidance behavior of rats with septal lesions. J Comp Physiol Psychol 1975;88:271-80.

3. Květňanský R, Dobrakovová M, Ježová D, Opešálová Z, Minarčík M, Golda V, Bárta J, Postel J. Ontogeny of mechanisms. GR van Loon, R.Květňanský, R.McCarty, Wistar strain - the results presented by Heofoo (1). This author studied the basal glycemia in the males of Wistar as well. He removed the blood samples from tail veins. No more details are given in the paper mentioned above. He described three experiments with the following basal glycemia levels: 4.18±0.22 (n=7), 3.81±0.17 (n=10), and 3.80±0.16 (n=7). The data suggest that intergroup differences can represented about 0.2 mol/l. About the repeated narcotization with ether. Our data suggest that repeated application of ether shows the stress effect (in our case the elevation of glycemia) in dependance on the frequency of an exposition the animal to ether vapor. When the intersampling intervals are 30 sec or more, we can see that the level of glycemia is equal or near to basal glycemia (when sampling was made under the condition of starvation) or to the first sample (when sampling was made in the animal with free access to pellets (see Table 2). No augmentation in stress reaction can be found. No differences were found when sampling was performed under ether and/or Nembutal anaesthesia (Table 2, the upper two rows. As to the pentobarbital anaesthesia (see in our experiments Nembutal), Moss (6) found that this type of narcotic did not alter any ACTH levels.

On the other hand, when the intersampling intervals are 2 sec, then there can be observed modified in the elaboration of stress reaction in the form of elevated glycemia (see Table 3). But is apparent that the augmentation of stress reaction is highly state dependent, i.e., the augmentation is twofold higher in the animals which was exposed to the starvation. Where is a proper reason of the mentioned differences it is not clear. It cannot be a priori excluded that the different state of available glycogen deposits can be judged as a main intervening variable.

This paper was supported By Internal Grant Agency of Ministry of Health of the Czech Republic No 5684-3. The authors wish to thank Carl T. Hansen, Animal Genetics Division, National Institute of Health, Bethesda, USA, for providing the genetically hypertensive rats of Koleczy type.

**Acknowledgement**

1. Moss IR, Vettori G, Golda CS. Institute of Experimental Neurosurgery, Charles University, Faculty of Medicine and Teaching Hospital, 500 05 Hradec Králové, Czech Republic.