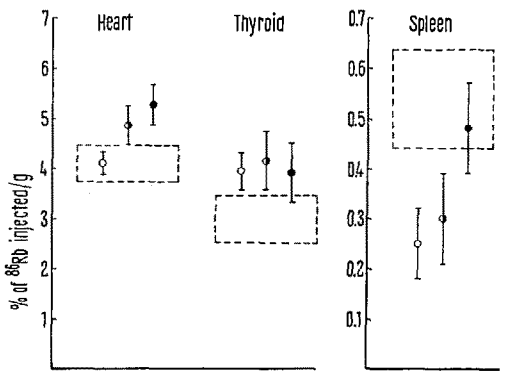
non-specific effect of the highly alkaline solution (pH 11.6) of diazoxide.

The effect of 5 mg diazoxide, injected i.v. within 30 sec, on blood pressure was determined in 5 rats. Ten seconds after starting the application the blood pressure fell rapidly, within 60 sec it reached minimum values (50% of control values), 5 min after the application the blood pressure was still considerably decreased (65% of control values).

The fall of the blood flow through the spleen is probably due to the contraction of this organ, caused by a rapid decline of the blood pressure[10]. The increase of the blood flow through the heart is consistent with the results of other authors[1,4]. Of interest is the increase in the thyroid gland, which has not been reported before. It is noteworthy that, as we were able to show, the 4 h uptake of radioactive iodine ^{131}I in the thyroid gland of rats is, after 5 mg of diazoxide, significantly inhibited[11]. The blood flow through the pancreas, the secretory function of which is supposed to be influenced by diazoxide[12], remains unchanged.

From the results presented it is possible to conclude that the immediate vascular effect of diazoxide is not the same in all vascular beds. The blood flow increases, in comparison with other organs, markedly only in the heart and thyroid.



Changes in local blood flow in the heart, thyroid gland and spleen of rats after i.v. injection of 5 mg of diazoxide. Mean values of the tissue uptake of ^{86}Rb expressed in % of injected dose/g of the tissue at the following time intervals between diazoxide and Rb uptake determination: ○ 5 sec, ◑ 90 sec, ● 150 sec. Verticals: 95% confidence intervals. Dashed lines: 95% confidence intervals of the control mean values.

Zusammenfassung. Der Einfluss von Diazoxid auf die Organdurchblutung der Ratten wurde mittels ^{86}Rb untersucht. Eine signifikante Durchblutungssteigerung des Herzens (128% der Kontrollwerte) und der Schilddrüse (139%) konnte bis 150 sec nach i.v. Injektion von 5 mg Diazoxid nachgewiesen werden. Die Durchblutung der Milz hingegen sank rasch auf 45% der Kontrollwerte, wobei nach 150 sec Normalisierung eintrat.

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Laboratory for Endocrinology and Metabolism, Charles University, Faculty of Medicine, Praha (Czechoslovakia), 6 September 1967.

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The Effect of Adrenaline on Different States of Sleep

It has been known that adrenaline, injected into the carotid artery, or the subarachnoidal space, or the lateral ventricle of the cat, produces 'anaesthesia-like' or 'sleep-like' effects[1-3]. Data about adrenaline influence on the quantity and quality of sleep, especially its paradoxical phase, are still missing. Therefore, the aim of this study was to investigate the effect of adrenaline on slow wave and paradoxical sleep.

Six adult cats (2.6–3.2 kg) were stereotaxically implanted with cortical monopolar and deep bipolar electrodes. Permanent cannula for intraventricular injections was inserted into right lateral ventricle. The animals were deprived of paradoxical sleep by the method of Jouvet[4] et al. for 3 days and nights. After 3 days and nights they were put into a soundproof chamber and their EEG were recorded, by Alvar VIII channel electroencephalograph, for 6 h. The cats were then allowed to rest for 10 days and the deprivation procedure of the same duration was repeated. The animals were again put into the soundproof chamber and after the first episode of paradoxical sleep appeared, adrenaline hydrochloride was administered intraventricularly. The % of wakefulness, slow sleep and paradoxical sleep was calculated from EEG-records.

The results obtained indicate an adrenaline action in 2 directions: suppression of paradoxical sleep, and promotion of wakefulness. The only component in sleep-wakefulness cycle which was not affected was slow wave sleep. This is shown in the Figure. In control experiments the quantity of paradoxical sleep (23.0 ± 4.9%) was, after intraventricular injection of adrenaline (0.2–2.0 mg), reduced (8.0 ± 4.4%). The EEG-pattern of paradoxical sleep after intraventricular administration of adrenaline remained unaltered. The episodes of paradoxical sleep usually occurred in the middle and in the second half of the recording time. The duration of the episodes of the paradoxical sleep was similar to those in control experiments. The increase of wakefulness (58.0 ± 11.8%) after intraventricular administration of adrenaline compared

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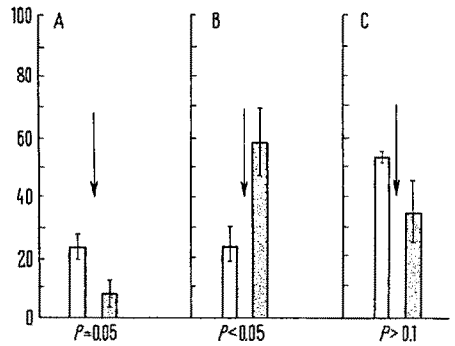
with control mean value (23.5 ± 6.6%) was dominant in all animals. Highly expressed desynchronization followed each injection of adrenaline and desynchronizing periods were present through the 6 h recuperative recording period. Their intensity and duration varied, although in 4 out of 6 animals they were most vividly expressed and longest at the beginning of the recording immediately after adrenaline injection. The other animals showed long waking periods in the middle and in the second half of the 6 h recording time. Slow wave sleep was also affected by the action of adrenaline, but not significantly (53.5 ± 1.6% in control experiments and 34.0 ± 9.4% after adrenaline). Periods of the slow sleep were present during the whole time of the recording, except immediately after intraventricular injection of adrenaline when desynchronization was dominant.



The action of adrenaline on different states of sleep in cats. Ordinate: columns in (A), paradoxical sleep in % of total time; columns in (B), wakefulness in % of total time; columns in (C), slow wave sleep in % of total time. Unfilled columns represent the mean values of 6 control experiments with its S.E.M. Columns with dots represent the mean values of 6 experiments with its S.E.M. after intraventricular injections of adrenaline. The arrow in (A), (B) and (C) denotes 10 days rest.

The dissociation of adrenaline activity exerted upon the duration of slow wave and paradoxical sleep contributes to the research findings which have shown that the sleep is not a homogenous state of behaviour. This dissociation would speak in favour of the dissociation of the origin of the 2 states of sleep where the drug target action site would be the brain stem structures underlying the mechanisms of vigilance and paradoxical sleep[5].

Résumé. On a étudié l'action de l'adrénaline sur les différents états de sommeil chez le chat. Pendant les premières 6 h de récupération après la privation de phase paradoxal du sommeil, l'injection intraventriculaire d'adrenaline (0.2–2.0 mg) diminue la phase paradoxale du sommeil et prolonge l'état de veille.

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2,4-Dinitrophenol Inhibition of P^{32} Release from Human Red Cells

We have previously reported[1] that the release of radioactive sulfate from labeled human erythrocytes can be inhibited by certain agents which uncouple oxidative phosphorylation in liver mitochondria, such as 2,4-dinitrophenol (DNP). This observation interests us because further investigation may reveal the nature of metabolic events at the membrane that may affect transport or permeability to various solutes. This report will show that $5 \times 10^{-4} M$ DNP also inhibits the release of radioactive phosphate (P^{32}) from human red cells and that the effect is more clearly observed when medium P_i concentration is elevated.

Fresh blood was collected in the usual mixture of acid, citrate, and dextrose (used in blood storage) and centrifuged at 800 *g* and 5 °C for 5 min. After removal of the plasma and buffy coat, the cells were washed 4 times with a modified Ringer-Locke's medium. This salt solution contained no calcium and was buffered with *Tris*(hydroxymethyl)aminomethane adjusted to pH 7.4 at 37 °C. The washed cells were suspended in an equal volume of this medium and labeled by incubating the suspension for 2 h at 37 °C in a Dubnoff shaking incubator, in the presence of 0.5 μC of P_i^{32}/ml of medium and 1 mM P_i. The labeled cells were washed twice and resuspended with sufficient non-radioactive medium to yield a hematocrit of 20%.

For the study of P^{32} release, 3.5 ml samples of the labeled blood suspension were placed in 25 ml Erlenmeyer flasks. These samples were removed after 10 and 130 min of incubation and aliquots of the suspension and of the medium were plated on concentrically-ringed aluminum planchets and air-dried. The radioactivity was counted with an end-window Geiger-Mueller counter and the results were expressed as % P^{32} released in 2 h. There was no significant change noted in the pH and hematocrit. When medium P_i levels were varied, appropriate amounts of NaCl were withheld to maintain iso-osmolality.

It may be noted in the Figure that the influence of DNP seems to be virtually absent when the experiment is conducted in a P_i-free medium. At a medium P_i concentration which corresponds to the usual plasma level (1 mM), a clear difference is noted with $10^{-3} M$ DNP but the change with $5 \times 10^{-4} M$ DNP is relatively small. At higher external P_i concentrations, there seems to be no doubt that both DNP levels inhibit P^{32} release. On the other hand, $10^{-4} M$ DNP has no noticeable effect at any medium P_i concentration.

We have noted before that augmentation of the medium P_i level results in an increase in P^{32} release[2]. This effect appears to be best explained by an internal metabolic

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