Parenteral feeding abolishes the circadian adrenocortical rhythm in rats

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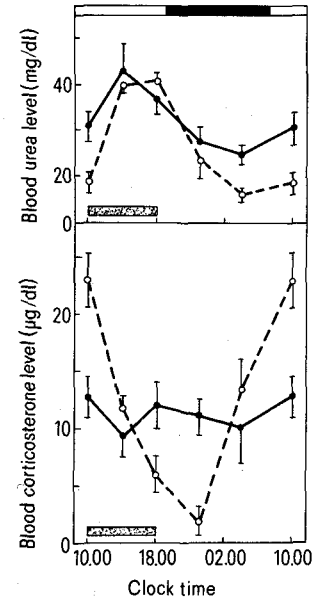
Summary. The circadian rhythm of the blood corticosterone level disappeared in rats that had been given a liquid diet i.v. during a resticted time of day for 10 days, suggesting an important role of the oro-gastrointestinal tract in formation of the rhythm.

Circadian rhythmicity in adrenocortical activity has been well documented in human subjects and laboratory animals. Recently, many workers[1-4] reported that the phase of the rhythmic changes in plasma corticosterone level in rats shifted when the feeding time was changed and that the peak of the corticosterone level appeared just before the feeding time, regardless of the lighting conditions. Furthermore, we found that it took about 10 days to establish the corticosterone rhythm corresponding to the feeding time, but once it had been established, it persisted during at least 2 days of starvation[5]. These results indicate that the circadian corticosterone rhythm in rats is endogenous, and that it is entrained by cycles of eating and fasting rather than by those of light and dark. However, little information is available as to where or how the effect of food intake acts as an entraining signal on the coricosterone rhythm. In the present study, we examined the corticosterone rhythm in rats kept under a parental feeding condition to elucidate the role of the sense of food in the oro-gastrointestinal tract in setting up the rhythm. For comparison with the corticosterone rhythm, we also examined the circadian rhythm of the blood urea level, which is known to be cued by time of feeding[6].

Methods. Male Wistar rats (200–240 g) were anesthetized by pentobarbital (40 mg/kg b.wt, i.p.), and a permanent cardiac catheter was implanted by passing a silicon tube (inner diameter 0.5 mm, outer diameter, 1.0 mm) through the jugular vein into the auricle by the method of Steffens[7]. The rats were kept in individual cages in an air-conditioned room at 24±2 °C with 12-h periods of light (lights on at 07.00 h and off at 19.00 h, LD). On the next day, they were divided into 2 groups, one for parenteral feeding and the other for oral feeding. In the parenteral feeding group, the catheter was connected to an infusion pump by an appropriate extension tube, and 28 ml of a liquid diet was infused at a constant rate of 3.5 ml/h during a restricted time of day (from 10.00 h to 18.00 h). The liquid diet consisted of 25 g/100 ml glucose, 8 g/100 ml amino acids, 35 mM NaCl, 20 mM K-acetate, 4 mM $MgSO_4$, 6 mM NaH_2PO_4 and 6 mM Ca-gluconate. In the oral feeding group, 28 ml of the same liquid diet was given in a feeding cup from 10.00 h to 18.00 h. To prevent the rats from eating all the diet during the first few hours, half was presented at 10.00 h and the remainder at 14.00 h. Both groups were allowed free access to water. After 10 days under these conditions of feeding, blood samples were obtained at 4–6 h intervals over a 24-h period from the tail vein of individual rats as described previously[5]. The concentration of blood corticosterone was determined by the protein-binding method of Murphy[8] with slight modifications as described previously[5]. The concentration of blood urea was determined with a urease kit (Urea Color Test, Boehringer-Mannheim, BRD).

Results and discussion. As reported previously[5], when rats are kept under LD and fed on a commercial laboratory chow during a restricted period in daytime for 10 days, blood corticosterone shows clear circadian rhythmic changes with a peak just before the feeding time. In the present study, we kept rats under LD and gave them a liquid diet p.o. or i.v. during a restricted time of day (10.00–18.00 h). The rats weighed 215±3 g (mean±SE, n=12) at the start of the experiments, and after 10 days under the oral and the parenteral feeding conditions they weighed 190±4 g (n=6) and 186±7 g (n=6), respectively. When the rats were fed orally, the blood corticosterone level showed clear circadian rhythmic changes, with a peak at 10.00 h, as shown in the figure. The blood urea level also showed circadian rhythmic changes with higher levels during the feeding time. These patterns of the rhythm were essentially the same as those of rats fed on a laboratory chow during the daytime[5,6]. When the rats were given the same liquid diet i.v., the blood urea levels were similar to those of rats fed p.o. and showed rhythmic changes with higher levels during the infusion time. In contrast, the blood corticosterone level remained at a rather constant level throughout the day and did not show any consistent rhythmic change. Thus, the parenteral feeding had no marked effect on the blood urea rhythm, but completely abolished the blood corticosterone rhythm.

We demonstrated previously[6] that the rhythmic rise and fall in the blood urea level are direct consequences of feeding and fasting, respectively: that is, the blood urea rhythm is an exogenous one. The present finding seems to agree well with this view, and thus, formation of the urea rhythm corresponding to the feeding time is independent of the route of administration of the diet. In contrast to this, the blood corticosterone rhythm is endogenous, and food presentation acts as an entrainer[5]. The most interesting finding



Circadian rhythms in the blood levels of urea (upper) and corticosterone (lower) in rats given a liquid diet p.o. (○----○) or i.v. (●——●). ▨, Times of diet restriction; ▭, times of light; ▬, times of dark. Points are the mean ± SEM for 6 rats.

in the present study was that the parenteral feeding abolished the corticosterone rhythm. This was not due to a nonspecific effect of the liquid diet used, because rats fed on the same diet orally showed a clear rhythm corresponding to the feeding time. It seems also possible that the chronic i.v. infusion had some nonspecific effects on the adrenocortical activity, but this is not likely because the daily mean level of blood corticosterone was roughly equal in the orally and the i.v. fed rats. Therefore, oral feeding itself appears to be an important factor for establishing the corticosterone rhythm.

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A negative inotropic effect of acetylcholine in the presence of several phosphodiesterase inhibitors

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Summary. The phosphodiesterase inhibitors papaverine, theophylline and 3-isobutyl-1-methylxanthine (IBMX) reveal a negative inotropic effect of acetylcholine in cat ventricular heart muscle. This effect is unrelated to β-adrenoceptor stimulation and possibly mediated by the accumulation of cyclic GMP.

The regulation by cholinergic stimulation of the cyclic GMP content was first suggested by George et al.[3] in 1970. Since then, many reports in the literature have appeared which both support and vitiate the hypothesis that cyclic GMP may help to mediate the effects of acetylcholine (ACh) on the heart[4,5]. A 2nd messenger function of cyclic GMP in the heart is strongly validated if the physiological effects of ACh are enhanced when the breakdown of the cyclic nucleotide is inhibited. We report here that the phosphodiesterase inhibitors papaverine, theophylline and IBMX[6] reveal a negative inotropic effect of ACh in cat ventricular heart muscle which is normally not responsive.

Cats were anaesthetized with ether and papillary muscles or left and right atria were dissected from the heart and mounted in a muscle chamber for recording electrical and/or mechanical activity as described earlier[7]. The preparations were driven electrically at 0.2 or 1.0 Hz. Drugs were freshly dissolved and added to the muscle chamber containing Tyrode's solution (composition in mM: NaCl 136.9; KCl 5.4; $MgCl_2$ 1.05; $CaCl_2$ 1.8; NaH_2PO_4 0.42; $NaHCO_3$ 11.9; glucose 5.6) which was gassed with 95% O_2–5% CO_2 and kept at 35 °C. Isoprenaline in test solutions was protected from oxidative degradation by ascorbic acid (50 mg/l) and EDTA (18.6 mg/l).

Figure 1 shows that ACh 10^{-5} M exerted virtually no effects on the force of contraction in cat ventricular heart muscle. The concentration of ACh 10^{-5} M corresponds to an EC_{95} in cat atrial muscle in the same experimental conditions (n = 110; not shown). Figure 1 further demonstrates that the force of contraction in cat papillary muscles was increased to about twice the amount of the control in the presence of isoprenaline 3×10^{-7} M. When ACh was then added, the positive inotropic response to isoprenaline was diminished, yet less so in the presence of atropine 10^{-6} M. The antagonism between cholinergic and adrenergic stimuli in the ventricle is well known[8] and has been ascribed to a modulation by muscarinic receptors of adenylate cyclase activation[9]. It is in line with this interpretation that in the presence of both cholinergic and adrenergic stimuli the force of contraction in the ventricle is always above the control level. However, in the presence of papaverine or theophylline or IBMX the force of contraction can be depressed by ACh to values below the control, which reflects a direct negative inotropic action of ACh on ventricular heart muscle unrelated to β-adrenergic stimulation.

In the present study, papaverine was preferentially used, since the effects of this drug on the force of contraction are relatively small in cat ventricular heart muscle[7]. Papaverine is also known to have an effect on intracellular calcium being released from mitochondria[10]. However, independent of a positive or a negative inotropic effect of papaverine, a concentration-dependent negative inotropic effect of the drug was seen in the presence of ACh 10^{-5} M. This probably means that a graded inhibition of the phosphodiesterase activity reveals a negative inotropic effect of ACh on mammalian ventricular heart muscle which is

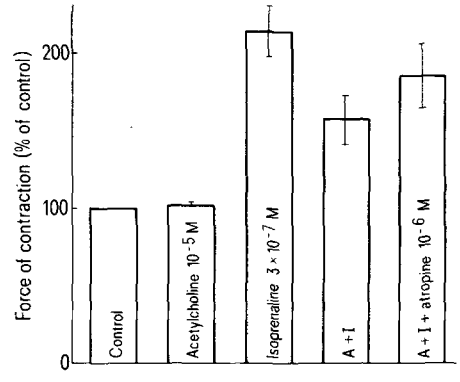

Fig. 1. Effects of ACh (A), isoprenaline (I), atropine and combinations of these drugs on the force of contraction in cat papillary muscles. After an equilibration period of 60–90 min the preparations were treated in the following sequence: 1. ACh 10^{-5} M for 15 min. 2. Drug-free Tyrode's solution for 30 min. 3. Isoprenaline 3×10^{-7} M (recording period 15 min). 4. Addition of ACh 10^{-5} M (recording period 15 min). 5. Addition of atropine 10^{-6} M (recording period 30 min). Frequency of stimulation: 1 Hz. Means ± SE of 8 preparations (paired data).