7 Butler, J.M., and Hammond, B.R., Br. J. Pharmac. *69* (1980) 495.
8 Stjernschantz, J., Sears, M., and Stjernschantz, L., Invest. Ophthal. Vis. Sci. *20* (1981) 53.
9 Bito, L.Z., Nichols, R.R., and Baroody, R.A., Exp. Eye Res. *34* (1982) 325.
10 Dyster-Aas, K., and Krakau, C.E.T., Invest. Ophthal. *3* (1964) 127.
11 Butler, J.M., Unger, W.G., and Cole, D.F., Quart. J. expt. Physiol. *65* (1980) 261.
12 Butler, J.M., and Hammond, B.R., Trans. ophthal. Soc. U.K. *97* (1977) 668.
13 Zhang, S.Q., Butler, J.M., Ohara, K., and Cole, D.F., Exp. Eye Res. *35* (1982) 43.
14 Holmdahl, G., Håkanson, R., Leander, S., Rosell, S., Folkers, K., and Sundler, F., Science *214* (1981) 1029.
15 Bynke, G., Håkanson, R., Hörig, J., and Leander, S., Eur. J. Pharmac. (1983) in press.
16 Bengtsson, E., Krakau, C.E.T., and Öhman, R., Invest. Ophthal. *14* (1975) 306.
17 Anjou, C.I.N., and Krakau, C.E.T., Acta ophthal. *39* (1961) 1.
18 Kao, C.Y., Pharm. Rev. *18* (1966) 997.
19 Folkers, K., Hörig, J., Rosell, S., and Björkroth, U., Acta physiol. scand. *111* (1981) 505.
20 Rosell, S., Björkroth, U., Hörig, J., Xu, J.-C., and Folkers, K., 8th int. Congr. Pharm. Tokyo (1983) abstract, in press.
21 Leander, S., Håkanson, R., Rosell, S., Folkers, K., Sundler, F., and Tornqvist, K., Nature, Lond. *294* (1981) 467.
22 Håkanson, R., Hörig, J., and Leander, S., Br. J. Pharmac. *77* (1982) 697.
23 Bill, A., Stjernschantz, J., Mandahl, A., Brodin, E., and Nilsson, G., Acta physiol. scand. *106* (1979) 371.
24 Otsuka, M., and Konishi, S., in: Substance P, p.207. Eds U.S. von Euler and B. Pernow. Raven Press, New York 1977.
25 Olgart, L., Gazelius, B., Brodin, E., and Nilsson, G., Acta physiol. scand. *101* (1977) 510.
26 Brodin, E., Gazelius, B., Lundberg, J.M., and Olgart, L., Acta physiol. scand. *111* (1981) 501.
27 Tervo, K., Tervo, T., Eränkö, L., Eränkö, O., and Cuello, A., Histochem. J. *13* (1981) 435.
28 Butler, J.M., Powell, D., and Unger, W.G., Exp. Eye Res. *30* (1980) 311.
29 Unger, W.G., Butler, J.M., Cole, D.F., Bloom, S.R., and McGregor, G.P., Exp. Eye Res. *32* (1981) 797.
30 Jessell, T.M., Iversen, L.L., and Cuello, A.C., Brain Res. *152* (1978) 183.
31 Bynke, G., Invest. Ophthal. (1983) in press.

0014-4754/83/090996-03$1.50 + 0.20/0

Differences in the content and turnover of noradrenaline in rat heart atria and ventricles and circadian-phase-dependency[1]

B. Lemmer and R. Weimer

Zentrum der Pharmakologie, J. W. Goethe-Universität, Theodor-Stern-Kai 7, D-6000 Frankfurt/Main 70 (Federal Republic of Germany), October 25, 1982

Summary. In light-dark-synchronized male rats the levels of noradrenaline in heart atria were about 3 times that found in heart ventricles. Noradrenaline turnover rate which were about 8–9 fold greater for the atria than for the ventricles displayed a circadian-phase-dependency with increased rates in the dark period in both parts of the rat heart.

There is sound evidence demonstrating circadian variations in the level of various biogenic amines in different organs, especially the brains of mammals[2–5]. However, the endogenous level is in fact the resultant of a number of underlying phenomena: synthesis, storage, release and inactivation of the amine, and may not be a representative measurement of the dynamics of the respective transmitter substance. The turnover of a biogenic amine reflects its level of functional activity in a much better way[5–7]. Previous studies in light-dark-synchronized male rats have shown that the turnover rate of the cardiac noradrenaline was significantly greater in the activity period of the night-active animals, i.e. in the dark period, than in the resting period during light, even though the level of noradrenaline in whole hearts did not vary with the time of the day[7–9]. That this circadian ryhthm was triggered by central nervous activity was demonstrated in experiments in which ganglionic transmission was blocked by chlorisondamine, which abolished the rhythm in the turnover of the cardic noradrenaline[8].

It is well known that the neurones of the cardiac sympathetic nervous system terminate mainly in structures of the heart atria. Since no data were available concerning the noradrenaline turnover rate and its circadian-phase-dependency in different parts of the rat heart, it was the aim of this investigation to study separately the concentration and the turnover of noradrenaline in the atria and ventricles of the heart at different times of the day. In previous studies the turnover of cardiac noradrenaline was studied by inhibiting either the tyrosine-hydroxylase[7] or after injection of ^{3}H(–)-noradrenaline[7–9]. Since in the present experiment inhibition of the dopamine-β-hydroxylase by FLA 63 was used to determine the noradrenaline turnover, the results obtained under identical experimental conditions with both nonradioactive and radioactive techniques could be compared.

Material and methods. Male Wistar rats (TNO W. 74) of about 130–160 g were used. The animals were kept for at least 7 days under a controlled lighting schedule of 12 h light (07.00–19.00 h, 200 lx) alternating with 12 h darkness (19.00–07.00 H < 0.1 lx) with food and water ad libitum and at a room temperature of 23 ± 1 °C.

The noradrenaline contents of the atria and ventricles of the rat heart were determined by a spectrofluorometric method[10] using the procedures described by Schlumpf et al.[11] and Chang[12] with slight modifications. Briefly, the modifications mainly involved the extension of the miniaturized method of Schlumpf et al.[11] for 1.5–5 mg samples to tissue amounts of 15–250 mg (for details see Weimer[10]).

The turnover of noradrenaline in atria and ventricles was determined separately either during the light period (L) or the dark period (D). The parameters of the turnovers ($t^1/_2$ = half-life, k = rate constant, turnover rate) were determined from the logarithmic decline of the endogenous noradrenaline content after inhibition of the dopamine-β-hydroxylase with FLA 63 (bis-[4-methyl-1-homopiperazinyl thiocarbonyl]-disulfid, Labkemi AB, Göteborg, Sweden). FLA 63 has been shown to be a potent inhibitor of dopamine-β-hydroxylase[13], leading to a rapid and selective

depletion of central and cardiac noradrenaline stores without affecting dopamine and serotonin stores[14].

Since earlier studies in whole hearts of rats, in which measurements were made at 3-h intervals during 24 h, had revealed no circadian variations in the endogenous cardiac noradrenaline level[7,15], the noradrenaline content in atria and ventricles of control animals was determined only at 2 different times of the day, i.e. at 07.30 h or at 19.30 h. At the same time points, groups of 5 rats were injected with FLA 63 (40 mg/kg, s.c.) and these animals were killed 4 h later. Thus, one turnover experiment during either L or D consisted of 5 control rats killed at 07.30 h or at 19.30 h and 5 FLA 63 injected rats killed 4 h later. The parameters of the turnover were calculated from 2-point-regression lines. This procedure was chosen in order to save animals, since preliminary experiments on rat heart at 0, 2 and 4 h after FLA 63, as well as recently-published similar experiments in rat brain[5] showed a log-linear decline in the noradrenaline content after administration of this compound.

After decapitation of the animals the hearts (total mean wet wt 540 mg) were dissected out and rinsed in ice-cold saline solution. Then the 2 atria (mean wet wt 20 mg) and a 200–250 mg portion of the apex of the ventricles were prepared and stored in liquid nitrogen until determinations were carried out on the next day. Significance between mean values was tested by the unpaired 2-tailed Student's t-test. Since the distribution of the half-lives of experiments within 1 group is asymmetric, the geometric means with the respective 95% confidence limits were calculated.

Results. Table 1 shows that the endogenous noradrenaline level is unevenly distributed in atria and ventricles of the rat heart. In heart atria the noradrenaline content is about 3 times higher than in the ventricles. The mean amine levels in either part of the rat heart, however, were not different at the 2 different circadian stages. On the basis of the corresponding tissue proportions, the total cardiac noradrenaline content is about 0.99 μg/g, as can be calculated from table 1. Considering that only the apex cordis was investigated in the present study this value is in good correspondence with the noradrenaline content of whole rat heats with mean values of 0.87–0.94 μg/g as found in several earlier studies in the same strain of rats[7-9].

The differences in the turnover of noradrenaline between atria and ventricles were even more pronounced than the differences in the endogenous amine levels. The parameters of the turnover calculated from 3 to 5 independent experiments performed at 2 different times of the day are summarized in table 2. In either period, the turnover rate of noradrenaline was about 8–9-fold greater in the atria than in the ventricles. Furthermore, in contrast to the endogenous noradrenaline level its turnover rate in either part of the heart displayed a significant temporal dependency with an approximately 2-fold greater turnover rate during D than during L. The relative increase in the noradrenaline turnover in the activity period of the rats, i.e. during D, however, was not different for the atria and the ventricles.

Discussion. The experimental results obtained in light-dark-synchronized rats clearly demonstrate for the first time that in the atria of the rat heart the steady state level and the turnover of the physiological transmitter noradrenaline are markedly higher than in the heart ventricles. Furthermore, the results extend recent findings in which it has been shown that the turnover of noradrenaline determined in whole rat hearts displays a temporal dependency, with increased turnover rates in the dark period[7-9]. However, in those experiments the turnover of noradrenaline was determined either after inhibition of the tyrosine hydroxylase with H 44/68[7] or after injection of ^{3}H-(-)-noradrenaline[7-9]. Most interestingly, with both the nonradioactive and the radioactive techniques, similar results were obtained in that the noradrenaline turnover rate was significantly greater during D than during L[7-9]. On the other hand, the absolute turnover rates obtained by the different methods yielded different results. The turnover rates of noradrenaline in whole hearts during L and D were 0.023 and 0.035 μg/g/h after H 44/68 and 0.043 and 0.087 μg/g/h after ^{3}H-(-)-noradrenaline[7]. On the basis of the corresponding tissue proportions the mean total turnover rates after FLA 63 amount to 0.023 and 0.044 μg/g/h during L and D, respectively. These values are in the same range as obtained after inhibition of the tyrosine-hydroxylase with H 44/68[7] (see above).

Since the experiments with the different techniques used to determine the cardiac noradrenaline turnover were performed in the same strain of male rats under identical

Table 1. Levels of noradrenaline ($\mu g \cdot g^{-1}$) in heart atria and heart ventricles of light-dark-synchronised rats at 2 different times of the day

Time of sacrifice	07.30 h	19.30 h
Atria	2.95 ± 0.06 (28)	3.00 ± 0.06 (55)
Ventricles	0.92 ± 0.03 (35)	0.90 ± 0.02 (68)

Mean values ± SEM of 28–68 heart atria and ventricles, respectively. Animals were sacrificed either at 07.30 h or at 19.30 h.

Table 2. Turnover of noradrenaline in rat heart atria and ventricles at different times of the day

	Light period			Dark period		
	Half-life $t_{1/2}$ (h)	Rate constant k (h^{-1})	Turnover rate ($\mu g \cdot g^{-1} \cdot h^{-1}$)	Half-life $t_{1/2}$ (h)	Rate constant k (h^{-1})	Turnover rate ($\mu g \cdot g^{-1} \cdot h^{-1}$)
Atria	17.2	0.0403	0.122	6.3	0.1098	0.331
	13.3	0.0520	0.155	6.9	0.1005	0.301
	10.4	0.0670	0.188	11.5	0.0601	0.158
				7.7	0.0900	0.280
$\bar{x} \pm$ SEM	13.1	0.0531	0.155	7.7	0.0901	0.270
	(9.3–22.1)[a]	± 0.0077	± 0.019	(5.4–13.1)[a]	± 0.0108[b]	± 0.038[b]
Ventricles	36.4	0.0190	0.018	13.8	0.0502	0.050
	38.4	0.0180	0.016	18.0	0.0385	0.035
	22.1	0.0311	0.016	20.8	0.0338	0.024
				25.1	0.0276	0.025
				15.2	0.0456	0.038
$\bar{x} \pm$ SEM	30.5	0.0227	0.017	17.7	0.0391	0.034
	(20.0–64.2)[a]	± 0.0042	± 0.007[c]	(12.5–30.2)[a]	± 0.0041[b]	± 0.005[b,c]

The parameters of the turnover were calculated from the decline of the endogenous noradrenaline content after inhibition of the amine synthesis with FLA 63 (40 mg/kg, s.c.). Mean values ± SEM of 3–5 experiments. [a] Geometric mean with 95% confidence limits, significance; [b] $p < 0.05$ between light period and dark period; [c] $p < 0.001$ between atria and ventricles.

conditions, the results demonstrate that at least the 2 nonradioactive techniques do not allow one to calculate 'absolute' turnover rates. Both inhibitors of the catecholamine synthesis also affect central monoamines by depleting dopamine and noradrenaline (H 44/68)[5,14-16] or noradrenaline (FLA 63)[5,13-16] stores. Furthermore, inhibition by H 44/68 leads to a decrease of the physiologically elevated motor activity in rats during D[17], and also antagonizes the central stimulation induced by amphetamine, dexamphetamine, H 77/77, L-dopa and various anticholinergic drugs[18-23]. Inhibition by FLA 63 of the stimulant properties of dexamphetamine, L-dopa and H 77/77 on motility was also described[19,22,24]. In naive mice, Svensson and Waldeck[25] reported a decrease in motor activity due to FLA 63 alone, whereas in rats this was not observed[17]. However, it has been shown that basal and drug-induced increased motility[26,27], as well as a drug-induced decrease in motility, are greatly dependent on the strain of rats and mice used[27,28]. Thus, these data clearly show that both H 44/68 and FLA 63, by depleting central catecholamine stores, obviously decrease cardiac sympathetic activity. This may well explain the lower turnover rate of cardiac noradrenaline both during L and D in comparison with the data obtained with the radioactive technique. Nevertheless, the experiments with FLA 63, in accordance with those obtained after H 44/68[7] and with ^{3}H-(-)-noradrenaline[7-9], clearly demonstrate that the turnover of noradrenaline in whole rat hearts as well as in heart atria and ventricles displays a circadian-phase-dependency.

1 Supported by grants of the Deutsche Forschungsgemeinschaft (Le 318/7, 318/8-2).

2 Friedman, A.H., and Walker, C.A., J. Physiol., Lond. *197* (1968) 77.
3 Quay, W.B., Gen. comp. Endocr. *3* (1963) 473.
4 Wurtman, R.J., and Axelrod, J., Life Sci. *5* (1966) 665.
5 Lemmer, B., and Berger, T., Naunyn-Schmiedeberg's Arch. Pharmak. *303* (1978) 257.
6 Costa, E., Biochem. Pharmac. *2* (1970) 169.
7 Lemmer, B., and Saller, R., Naunyn-Schmiedeberg's Arch. Pharmak. *282* (1974) 75.
8 Lemmer, B., and Wenda, K., Pharmac. biochem. Behav. *10* (1979) 361.
9 Lemmer, B., and Charrier, A., Naunyn-Schmiedeberg's Arch. Pharmak. *313* (1980) 205.
10 Weimer, R., thesis. University of Frankfurt, Frankfurt 1982.
11 Schlumpf, M., Lichtensteiger, W., Langemann, H., Waser, P.G., and Hefti, F., Biochem. Pharmac. *23* (1974) 2437.
12 Chang, C.C., J. Neuropharmac. *3* (1964) 643.
13 Florvall, L., and Corrodi, H., Acta pharmaceut. suec. *7* (1970) 7.
14 Corrodi, H., Fuxe, K., Hamberger, B., and Ljungdahl, Å., Eur. J. Pharmac. *12* (1970) 145.
15 Saller, R., thesis. University of Frankfurt, Frankfurt 1976.
16 Andén, N.-E., and Fuxe, K., Br. J. Pharmac. *43* (1971) 747.
17 Lemmer. B., and Berger, T., Naunyn-Schmiedeberg's Arch. Pharmak. *303* (1978) 251.
18 Weismann, E.D., and Koe, B.K., Life Sci. *4* (1965) 1037.
19 Svensson, T.H., Eur. J. Pharmac. *12* (1970) 161.
20 Thornburg, J.E., and Moore, K.E., Neuropharmacology *12* (1973) 853.
21 Thornburg, J.E., and Moore, K.E. Neuropharmacology *12* (1973) 1179.
22 Lassen, J.B., Psychopharmacologia, Berl. *37* (1974) 331.
23 Ahlenius, S., Brain Res. *69* (1974) 57.
24 Strömberg, U., and Svensson, T.H., Psychopharmacologia, Berl. *19* (1971) 53.
25 Svensson, T.H., and Waldeck, B., Eur. J. Pharmac. *7* (1969) 272.
26 Davis, W.M., Babbini, M., Pong, S.F., King, W.T., and White, C.L., Pharmac. biochem. Behav. *3* (1974) 803.
27 Lemmer, B., Caspari-Irving, G., and Weimer, R., Pharmac. biochem. Behav. *15* (1981) 173.
28 Anisman, H., and Kokkinidis, L., Psychopharmacologia, Berl. *45* (1972) 243.

0014-4754/83/090998-03$1.50 + 0.20/0

Voltage oscillations in mammalian metaphase II oocytes

F. Eusebi, G. Fratamico, R. Colonna and F. Mangia[1]

Institute of Histology and General Embryology, University of Rome, via A. Scarpa 14, I-00161 Roma (Italy), Department of Physics and INFN, University of Bologna, Bologna (Italy), and Institute of Biology and General Zoology, University of L'Aquila, L'Aquila (Italy), November 8, 1982

Summary. The membrane potential has been measured in ovulated mouse oocytes using conventional electrophysiological techniques. Temporal oscillations in membrane voltage have been observed in the oocytes, with periods of about 6 h. This oscillatory pattern, peculiar to oocytes in metaphase II, might explain the differences in membrane potential values reported in several studies on mammalian oocytes.

Biological oscillations span a wide range of frequencies and wave-like phenomena. Circadian rhythms in mammalian biological clocks for instance, measure periods in h, while biochemical oscillations tend to have periods of the order of min[2]. Oscillatory patterns of membrane potential with a shorter period (of the order of sec)[3-4], have been shown in vertebrate nerve cells. Wave-like oscillations of resting membrane potential (RP) with a period of about 6 h are here described in mouse oocytes in metaphase of the 2nd meiotic division.

Materials and methods. Egg collection. Unfertilized and fertilized eggs were obtained from the oviducts of unmated or mated superovulated mice (Swiss CD1). Superovulation was induced by i.p. gonadotropin injection[5]. The interval between human chorionic gonadotropin (hCG) injection and egg collection was between 12 an 24 h. The oocytes were cleared of cumulus cells by treatment with hyaluronidase, (300 IU ml^{-1}). Isolation and cell washing procedures were performed in MH, a HEPES-buffered oocyte culture medium with the following composition (mM); NaCl, 94.6; KCl, 4.8; $CaCl_2$, 1.7; $MgSO_4$, 1.2; KH_2PO_4, 1.2; Na-lactate, 23.3; Na-pyruvate, 0.33; glucose, 5.6. The medium was buffered with HEPES-NaOH, 25 mM. Following isolation, oocytes were immediately processed for electrophysiology. In some experiments, as indicated in the text, the oocytes were collected 12 h after hCG injection and cultured at 37 °C for various times before electrophysiological recordings were made.