6 Cattell, R.B., The effect of alcohol and caffeine on intelligent and associative performance. Br. J. med. Psych. *10* (1930) 20–33.
7 Curatolo, P., and Robertson, D., The health consequences of caffeine. Ann. int. Med. *98*/1 (1983) 641–653.
8 Davis, R.C., Buchwald, A.M., and Frankman, R.W., Autonomic and muscular responses and their relation to simple stimuli. Psych. Monogr. *69* (1955) 1–71.
9 Dews, P.B., Caffeine. Ann. Rev. Nutr. *2* (1982) 323–341.
10 Eichler, O., Kaffee und Coffein, 2nd edn. Springer-Verlag, Berlin 1976.
11 Estler, C.-J., Caffeine, in: Psychotropic Agents, Part III, Alcohol and Psychotomimetics, Psychotropic Effects of Central Acting Drugs, pp. 369–389. Eds F. Hoffmeister and G. Stille. Springer-Verlag, Berlin 1982.
12 Fahrenberg, J., Selg, H., and Hampel, R., Das Freiburger Persönlichkeitsinventar FPI, 3rd edn. Verlag für Psychologie Dr C.J. Hogrefe, Göttingen 1978.
13 File, S.E., Bond, A.J., and Lister, R.G., Interaction between effects of caffeine and lorazepam in performance tests and self-ratings. J. clin. Psychopharmac. *2* (1982) 102–106.
14 Flory, C.D., and Gilbert, J., The effects of benzedrine sulphate and caffeine citrate on the efficiency of college students. J. appl. Psychol. *27* (1943) 121–134.
15 Franks, H.M., Hagedorn, H., Hensley, V.R., Hensley, W.J., and Starmer, G.A., The effect of caffeine on human performance, alone and in combination with ethanol. Psychopharmacology *45* (1975) 177–181.
16 Gilbert, R.M., Caffeine as a drug of abuse, in: Research Advances in Alcohol and Drug Problems, vol. 3, pp. 49–176. Eds R.J. Gibbins, Y. Israel, H. Kalant, R.E. Popham, W. Schmidt and R.G. Smart. Wiley & Sons, New York 1976.
17 Gilliland, A.R., and Nelson, D., The effects of coffee on certain mental and physiological functions. J. gen. Psychol. *21* (1939) 339–348.
18 Hrbek, J., Komenda, S., Mačáková, J., and Široká, A., Acute effect of chlorprothixen (5 mg), caffeine (200 mg), and the combination of both drugs on verbal associations. Activitas nerv. sup. *13* (1971) 207–208.
19 Jick, H., Miettinen, O.S., Neff, R.K., Shapiro, S., Heinonen, O.P., and Slone, D., Coffee and myocardial infarction. New Engl. J. Med. *298* (1973) 63–67.
20 Knutti, R., Rothweiler, H., and Schlatter, C., Effect of pregnancy on the pharmacokinetics of caffeine. Eur. J. clin. Pharmac. *21* (1981) 121–126.
21 Lehmann, H.E., and Csank, J., Differential screening of phrenotropic agents in man: Psychophysiologic test data. J. clin. Psychopath. *18* (1957) 222–235.
22 Lienert, G.A., and Huber, H.P., Differential effects of coffee on speed and power tests. J. Psychol. *63* (1966) 269–274.
23 Life Sciences Research Office, Federation of American Societies for Experimental Biology, Evaluation of the health aspects of caffeine as a food ingredient, 1978. (NTIS No. PB-234984/4).
24 Mitchell, V.E., Ross, S., and Hurst, P.M., Drugs and placebos: Effects of caffeine on cognitive performance. Psychol. Rev. *35* (1974) 875–883.
25 Nash, H., Alcohol and Caffeine: A Study of Their Psychological Effects. Thomas, Springfield 1962.
26 Report from the Boston Collaborative Drug Surveillance Program, Coffee drinking and acute myocardial infarction. Lancet *2* (1972) 1278–1281.
27 Ritchie, J.M., Central nervous system stimulants. The xanthines, in: The Pharmacological Basis of Therapeutics, 5th edn, pp. 367–378. Eds L.S. Goodman and A. Gilman. Macmillan, New York 1975.
28 Spaiser, L.H., An infra-red photoplethysmograph coupler. Psychophysiology *14* (1977) 75–77.
29 Stimmel, B., Cardiovascular Effects of Mood-Altering Drugs. Raven Press, New York 1979.
30 Suter, T.W., Psychophysiological Effects of Cigarette Smoking and Responding to Conflict Inducing Stimuli in Habitual Smokers. Doctoral diss. ETH Zürich, No. 6839 (1981).
31 Suter, T.W., Feierabend, J.M., and Bättig, K., Physiologische Korrelate von zwei verschiedenartigen mentalen Tätigkeiten: Konfliktlösen vs Konzeptlernen. Schweiz. Z. Psychol. *3* (1982) 203–216.
32 Tahmoush, A.J., Jennings, J.R., Lee, A.L., Camp, S., and Weber, F., Characteristics of a light emitting diode – Transistor photoplethysmograph. Psychophysiology *13* (1976) 357–362.
33 Truitt, E.B., Jr, The xanthines, in: Drill's Pharmacology in Medicine, 4th ed., pp. 533–556. Ed. J.R. DiPalma. McGraw-Hill, New York 1971.
34 Weiss, B., and Laties, V.G., Enhancement of human performance by caffeine and the amphetamines. Pharmac. Rev. *14* (1962) 1–36.

0014-4754/84/111218-06$1.50 + 0.20/0

A pivotal role for serotonin (5HT) in the regulation of beta adrenoceptors by antidepressants: reversibility of the action of parachlorophenylalanine by 5-hydroxytroptophan[1]

D.H. Manier, D.D. Gillespie, L.R. Steranka[1] and F. Sulser

Tennessee Neuropsychiatric Institute and Vanderbilt University, School of Medicine, Department of Pharmacology, Nashville (Tennessee 37232, USA), 25 January 1984

Summary. An acute reduction in the synaptic availability of serotonin (5HT) by p-chlorophenlalanine (PCPA) nullifies the decrease in the density of cortical beta adrenoceptors caused by desipramine (DMI) but does not appreciably alter the attenuation of the norepinephrine (NE) sensitive adenylate cyclase. The analysis of competition-binding curves of [^{3}H]-dihydroalprenolol shows that the affinity of the agonist (–)-isoproterenol for cortical beta adrenoceptors is profoundly reduced following PCPA. This reduction in agonist affinity is enhanced by DMI. Resupplying 5HT by by-passing trptophan hydroxylase inhibition, by administering 5-hydroxytryptophan, converts a DMI non-responsive to a DMI responsive beta adrenoceptor population and shifts the markedly decreased agonist affinity towards the affinity values found in control preparations. The results demonstrate the pivotal role of 5HT in the regulation of the density and agonist affinity characteristics of cortical beta adrenoceptors and contribute to the scientific basis of the 'serotonin-norepinephrine link hypothesis' of affective disorders.
Key words. Cortical beta adrenoceptors; density; agonist affinity; role of serotonin; desipramine; p-chlorophenylalanine; 5-hydroxytryptophan.

Clinically effective antidepressant treatments – pharmacotherapy and ECT – cause, upon administration on a clinically relevant time basis, an attenuation of the norepinephrine (NE) sensitive adenylate cyclase in brain that is generally linked to a down-regulation of the density of beta adrenoceptors[22]. Experimental evidence

indicates that an unhindered synaptic availability of NE at the receptor is one prerequisite for desensitization by antidepressants of the NE sensitive adenylate cyclase system in brain[8, 12, 13, 17, 18, 24]. More recent studies have generated evidence for a co-requirement of an intact serotonergic neuronal input for down-regulation of beta adrenoceptors by tricyclic antidepressants[3, 5, 9].

The present studies were undertaken to ascertain whether a more acute change in the synaptic availability of serotonin (5HT) would interfere with antidepressant-induced changes in the density and the functional characteristics of cortical beta adrenoceptors and whether or not such changes are reversible. A rapid reversibility of beta adrenoceptor number and function in brain as a consequence of changes in the synaptic availability of 5HT would facilitate future studies on molecular mechanisms involved in the reduction of beta adrenoceptors during the process of homologous desensitization of this receptor-coupled adenylate cyclase system.

Methods

Male Sprague-Dawley rats (180–200 g) from Harlan Industries (Indianapolis, Indiana, USA) were used in all experiments. The animals were maintained under standard laboratory conditions with a 12-h light-dark cycle and with free access to water and pelleted standard rat chow. The status of beta adrenoceptors was determined according to Bylund and Snyder[4] using [^{3}H]-dihydro-alprenolol ([^{3}H]-DHA; New England Nuclear Corporation, Boston, Massachusetts, USA: specific activity approximately 45 Ci/mmole) as the ligand. Specific [^{3}H]-DHA binding represents total binding minus nonspecific binding in the presence of 10 μM d,l-propranolol. B_{max} and K_d values were determined by Scatchard analysis[16] using concentrations of [^{3}H]-DHA ranging from 0.25 to 5.0 nM. Competition-binding curves for inhibition of specific [^{3}H]-DHA binding by (−)-isoproterenol were constructed using a concentration of 2–3 nM [^{3}H]-DHA and (−)-isoproterenol over a range from 0.001 to 100 μM. The response of the NE sensitive adenylate cyclase was determined in slices of the rat cortex. The cortex was sliced with a McIlwain tissue chopper at 0.3-mm intervals in a single plane. The slices were incubated in Krebs-Ringer bicarbonate buffer (approximately 50 mg of tissue/ml buffer) at 37 °C, pH 7.4 with 95% O_2, 5% CO_2 being bubbled into each incubation vessel. After 30 min, the buffer was changed and after an additional 15 min of incubation, NE (100 μM) was added. 10 min after the addition of NE, the buffer solution was removed by aspiration and the slices were frozen by liquid nitrogen and homogenized in 3.5 ml of ice cold 0.3 M perchloric acid using a Polytron homogenizer. Cyclic AMP was isolated by ion exchange chromatography as previously described[2] and assayed according to Gilman[7]. Protein was determined in 0.5-ml aliquots of the perchloric acid homogenate according to Lowry et al.[10]. The biogenic amines, NE and 5HT, were analyzed using high pressure liquid chromatography with electrochemical detection as previously described in detail[14].

Results

To test whether an acute reduction in the synaptic availability of 5HT would interfere with DMI-induced changes in beta adrenoceptor number and function, we examined the consequences of tryptophan hydroxylase inhibition by p-chlorophenylalanine (PCPA) on the DMI induced down-regulation of beta adrenoceptors and the attenuation of the NE sensitive adenylate cyclase. As can be seen in the figure, PCPA (400 mg/kg i.p.) nullified within two days the DMI-induced decrease in the density of beta adrenoceptors but did not appreciably alter the attenuation of the NE sensitive adenylate cyclase. (400 mg PCPA/kg/day for two days reduced the concentration of 5HT in cortex from 236 ± 16 (14) to 25 ± 3 (15) ng/g while the concentration of NE declined from 248 ± 9 (10) to 171 ± 10 (9) ng/g.) The analysis of competition-binding curves reveals that cortical beta adrenoceptor preparations from PCPA-treated animals display a marked decrease in the affinity for the agonist (−)-isoproterenol. As in cortical tissue from animals with lesions due to 5,7-dihydroxytryptamine[11], this reduction in agonist affinity is accentuated in cortical membrane preparations from

Role of serotonin in the regulation of number and function of β-adrenoceptors by DMI

	[^{3}H]-DHA binding B_{max} (fmoles/mg protein ± SEM)		% control	K_d (nM ± SEM)	IC_{50} (−)-isoproterenol (μM ± SEM)	
A) Normal control						
Saline	180 ± 7	(12)	100	1.40 ± 0.11	0.40±0.03	(13)
DMI	116 ± 9	(10)[a]	64[a]	1.25 ± 0.14	0.44 ± 0.03	(6)
B) PCPA-treated						
Saline	238 ± 10	(4)[d]	100	1.49 ± 0.14	2.49 ± 0.54	(6)[d]
DMI	216 ± 12	(12) (NS)	91	2.14 ± 0.24[c]	7.48 ± 1.46	(7)[e]
5HTP + DMI	151 ± 3	(7)[b]	63[b]	1.93 ± 0.25	0.85 ± 0.14	(6)[b]

A: Control animals were given DMI (15 mg/kg twice daily for two days) and 15 mg/kg/day for an additional two days. The animals were sacrificed 24 h after the last dose of the drug and Scatchard analyses and competition binding curves performed as described under methods. The IC_{50} value designates the concentration of (−)-isoproterenol that displaces 50% of [^{3}H]-DHA.

B: Animals were given DMI as above. At day 3, PCPA (200 mg/kg/day) or PCPA + 5HTP (100 mg/kg/day) were administered for two days and the animals were sacrificed 24 h after the last dose of the drugs and the characteristics of beta adrenoceptors determined as described under methods. Numbers in parentheses indicate the number of animals.

[a] $p < 0.001$, DMI vs saline; [b] $p < 0.001$, 5HTP + DMI vs DMI; [c] $p < 0.05$, DMI vs saline; [d] $p < 0.005$, PCPA vs saline controls; [e] $p < 0.001$, DMI vs saline.

animals which were concomitantly treated with DMI. Thus, the IC_{50} value for (−)-isoproterenol increased from 0.40 ± 0.03 μM (saline) to 2.49 ± 0.54 μM (PCPA) to 7.48 ± 1.46 μM (PCPA+DMI) (table).

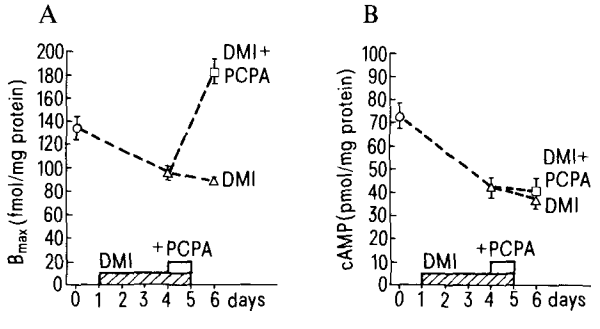
To delineate more precisely the role of 5HT in the regulation of density and agonist affinity of beta adrenoceptors, we aimed to restore the PCPA-induced decreases in synaptic availability of 5HT by administering 5-hydroxytryptophan (5HTP). The data in the table, B, demonstrate that such a procedure converts a DMI-nonresponsive to a DMI-responsive beta adrenoceptor population, i.e. DMI reduces the number of beta adrenoceptors to about the same extent as in saline treated control animals (63 and 64%, respectively, of corresponding control values). Moreover, the profound decrease in agonist affinity of beta adrenoceptors in PCPA-treated animals given DMI (IC_{50} 7.48 ± 1.46 μM) is also 'reversed' by 5HTP. The IC_{50} value of (−)-isoproterenol (0.85 ± 0.14 μM) now approaches affinity values of control preparations (table, A and B).

Discussion

Since PCPA, unlike the neurotoxin 5,7-dihydroxytryptamine, does not alter the concentration of substance P, or TRH[6] – peptides which may function as co-transmitters – the experimental data presented in this paper provide unequivocal evidence for the co-requirement of the synaptic availability of 5HT in the process of down-regulation of central beta adrenoceptors by DMI. It is noteworthy that the rather acute reduction in the synaptic availability of 5HT by PCPA nullified the DMI-induced down-regulation of beta adrenoceptors within two days despite the continuous administration of the tricyclic antidepressant, but did not reverse the attenuation of the NE sensitive adenylate cyclase. This dissociation between the density of beta adrenoceptors and the neurohormonal responsiveness is of interest as it emphasizes that ligand binding data do not necessarily reflect the functional status of a receptor system. The results indicate that a reduction in the synaptic availability of 5HT makes beta adrenoceptors not only 'non-regulatable' by DMI but somewhat less 'functional'. The slight but significant increase in the number of beta adrenoceptors following PCPA could be the consequence of the drug's effect on the concentration of NE.

Considering the marked changes in beta adrenoceptor characteristics following PCPA, it appears that beta adrenoceptor agonists are less potent under conditions of impaired serotonergic neuronal activity. It is important that resupplying 5HT by by-passing the tryptophan hydroxylase inhibition converted a DMI-resistant to a DMI-responsive beta adrenoceptor population and 'normalized' the profound decrease in agonist affinity for these receptors. Although the molecular basis of the regulation of density and affinity of beta adrenoceptors in brain remains to be elucidated, the demonstrated rapid reversibility of the number and characteristics of beta adrenoceptors as a function of the availability of 5HT provides an experimental opportunity to probe into molecular mechanisms of the fine regulation of NE receptor density and function. Since the DMI induced down-regulation of beta adrenoceptors is rapidly reversible in vivo, the apparent loss of receptors is more likely due to conformational alterations of the receptor in the phospholipid bilayer that distort the recognition site for the radioligand rather than to an actual loss, perhaps by internalization and lysosomal degradation, as has been described for a variety of peptide hormones[15]. With regard to agonist-induced desensitization of beta adrenoceptors in frog erythrocytes, Stadel et al.[21] have provided evidence that beta adrenoceptors are moved into a sequestered vesicle compartment from which they can recycle to the cell surface. An extrapolation from such in vitro models in erythrocytes to neuronal beta adrenoceptor systems in brain is, however, not possible at this time.

The demonstration that PCPA nullifies within two days the action of DMI on the density of beta adrenoceptors is of clinical interest as Shopsin et al.[19,20] have reported that PCPA nullifies within two days the therapeutic efficacy of tricyclic antidepressants and MAO inhibitors in patients with bipolar and unipolar depressions. Assuming that the delayed down-regulation of beta adrenoceptors by antidepressant treatments indeed reflects a therapeutically relevant biochemical action, the experimental results presented in this paper provide a scientific rationale for the clinical observations. Moreover, the present results generate support for the 'serotonin-norepinephrine link hypothesis' of affective disorders[23].



Effect of p-chlorophenylalanine (PCPA) on desipramine (DMI)-induced changes in density of β-adrenoceptors (A) and responsiveness of the norepinephrine (NE) sensitive adenylate cyclase (B). DMI was administered daily (10 mg/kg i.p. twice a day) for five days. PCPA (400 mg/kg i.p.) was administered for two days (day 4 and day 5). The rats were sacrificed 24 h after the last dose of the drugs. The cyclic AMP response represents the stimulated level of cyclic AMP (100 μM NE) minus the basal level which varied between 12.3 and 29.9 pmoles/mg protein. The number of animals in each experimental group varied from 4 to 8.

1 Acknowledgments. This work was supported by USPHS grant MH-29228 and the Tennessee Department of Mental Health and Mental Retardation. Present address of L.R. Steranka: NOVA Pharmaceutical Corporation, Baltimore (MD 21228, USA).

2 Blumberg, J.B., Vetulani, J., Stawarz, R.F., and Sulser, F., The noradrenergic cyclic AMP generating system in the limbic forebrain: Pharmacological characterization and possible role in the mode of action of antipsychotics. Eur. J. Pharmac. *37* (1976) 357–366.

3 Brunello, N., Barbaccia, M.L., Chuang, D.M., and Costa, E., Downregulation of β-adrenergic receptors following repeated injections of desmethylimipramine: Permissive role of serotonergic axons. Neuropharmacology *21* (1982) 1145–1149.

4 Bylund, D.B., and Snyder, S.H., Beta adrenergic receptor binding in membrane preparations from mammalian brain. Molec. Pharmac. *12* (1976) 568–580.
5 Dumbrille-Ross, A., and Tang, S.W., Noradrenergic and serotonergic input necessary for imipramine-induced changes in beta but not S_2 receptor densities. Psychiatry Res. *9* (1983) 207–215.
6 Gilbert, R.F.T., Bennett, G.W., Marsden, C.A., and Emson, P.C., The effects of 5-hydroxytryptamine depleting drugs on peptides in the ventral spinal cord. Eur. J. Pharmac. *76* (1981) 203–210.
7 Gilman, A.G., A protein binding assay for adenosine 3′,5′ cyclic monophosphate. Proc. natl. Acad. Sci. USA *67* (1970) 30ö5–312.
8 Janowsky, A., Steranka, L.R., Gillespie, D.D., and Sulser, F., Role of neuronal signal input in the down-regulation of central noradrenergic receptor function by antidepressant drugs. J. Neurochem. *39* (1982) 290–292.
9 Janowsky, A., Okada, F., Manier, D.H., Applegate, C.D., Steranka, L.R., and Sulser, F., Role of serotonergic input in the regulation of the β-adrenergic receptor coupled adenylate cyclase system. Science *218* (1982) 900–901.
10 Lowry, D.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., Protein measurement with the folin phenol reagent. J. biol. Chem. *193* (1951) 265–275.
11 Manier, D.H., Okada, F., Janowsky, A.J., Steranka, L.R., and Sulser, F., Serotonergic denervation changes binding characteristics of beta adrenoceptors in rat cortex. Eur. J. Pharmac. *86* (1983) 137–139.
12 Mishra, R., Gillespie, D.D., Lovell, R., Robson, R.D., and Sulser, F., Oxaprotiline: Induction of central noradrenergic subsensitivity by its (+)-enantiomer. Life Sci. *30* (1982) 1747–1755.
13 Mishra, R., Gillespie, D.D., Youdim, M., and Sulser, F., Selective MAO inhibition and noradrenergic receptor function in rat cortex. Psychopharmacology *81* (1983) 220–223.
14 Mishra, R., Leith, N.J., Steranka, L., and Sulser, F., The noradrenaline receptor coupled adenylate cyclase system in brain. Naunyn-Schmiedeberg's Arch. Pharmac. *316* (1981) 218–224.
15 Pastan, I.H., and Willingham, M.C., Journey to the center of the cell: Role of the receptosome. Science *214* (1981) 504–509.
16 Scatchard, G., The attractions of proteins for small molecules and ions. Ann. N.Y. Acad. Sci. *51* (1949) 660–672.
17 Schweitzer, J.W., Schwartz, R., and Friedhoff, A.J., Intact presynaptic terminals required for beta-adrenergic receptor regulation by desipramine. J. Neurochem. *33* (1979) 377–379.
18 Scott, J.A., and Crews, F.T., Rapid decrease in rat brain beta adrenergic receptor binding during combined antidepressant alpha$_2$ antagonist treatment. J. Pharmac. exp. Ther. *224* (1983) 640–646.
19 Shopsin, B., Friedman, E., and Gershon, S., The use of synthesis inhibitors in defining a role for biogenic amines during imipramine treatment in depressed patients. Psychopharmac. Commun. *1* (1975) 239–249.
20 Shopsin, B., Friedman, E., and Gershon, S., Parachlorophenylalanine reversal of tranylcypromine effects in depressed patients. Archs. gen. Psychiat. *33* (1976) 811–819.
21 Stadel, M.M., Strulovici, B., Nambi, P., Lavin, T.N., Briggs, M.M., Caron, M.G., and Lefkowitz, J., Desensitization of the β-adrenergic receptor of frog erythrocytes. J. biol. Chem. *258* (1983) 3032–3038.
22 Sulser, F., Noradrenergic receptor regulation and the action of antidepressants, in: Depression and Antidepressants – Recent Events, pp. 24–36. Excerpta Medica, Amsterdam 1983.
23 Sulser, F., The 'Serotonin-Noradrenaline Link Hypothesis' of affective disorders, in: Proc. IIIrd World Congress of Psychiatry. Plenum Press, New York 1984 (in press).
24 Wolfe, B.B., Harden, T.K., Sportn, J.R., and Molinoff, P.B., Presynaptic modulation of beta adrenergic receptors in rat cerebral cortex after treatment with antidepressants. J. Pharmac. exp. Ther. *207* (1978) 446–457.

0014-4754/84/111223-04$1.50 + 0.20/0

Sex differences in human circadian rhythms: Intrinsic periods and sleep fractions

by R.A. Wever

Max-Planck-Institut für Psychiatrie, Arbeitsgruppe Chronobiologie, D-8138 Andechs (Federal Republic of Germany), 30 August 1983

Summary. The period of freerunning circadian rhythms is significantly shorter and the fraction of sleep is significantly larger in human females than in males, as long as the rhythms run internally synchronized. The sex difference in the period could be a property either of the whole circadian system or of only one of the oscillators in a multi-oscillator system. The sex difference in the sleep fraction could be a fixed property of the sleep-wake rhythm or could depend on interactions in the multi-oscillator system. To investigate these questions, a sample of 33 long-term experiments, in which the rhythms ran internally synchronized in one section and internally desynchronized in another section, were analyzed. The periods of rhythms in rectal temperature were different in females and males during internal synchronization, but became identical during internal desynchronization. In contrast, sex differences in sleep-wake periods were more pronounced when the rhythms were desynchronized than when they were internally synchronized. This result provides evidence that the sex difference in periodicity is a property only of the sleep-wake rhythm; the intrinsic periods of temperature rhythms are identical in females and males, whereas those of sleep-wake rhythms are distinctly shorter in females than in males. In the state of internal synchronization, the joint period is a compromise between the intrinsic periods of the rhythms involved, and therefore it shows a small but significant sex difference. Moreover, the transition from internally synchronized to desynchronized rhythms is combined with a highly significant reduction in the sleep fraction, which is considerably greater in females than in males. These results suggest that the occurrence of internal desynchronization strongly affects the sleep-wake rhythm, and that the influence of rhythm disorders is considerably greater in females than in males.
Key words. Circadian rhythms; sex difference; sleep.