vated, eyes were open and some movements were observed. Heart rate throughout this stage oscillated between 23 and 49 beats/min ($\bar{X}$ = 40). EEG activity (fig. 1A) was of relatively high voltage and fast frequency (20–90 μv; 10–25 Hz). When animals were passing on to QW, the limbs were flexed and the shell rested on the cage floor; the neck was partially extended; eyes were usually open, with periodical closure preceding behavioral sleep. Heart rate slightly declined to an average of 32 ± 8 beats/min. EEG activity (fig. 1B) was similar to that of the preceding state. Each animal characteristically spent most of the 24-h period quietly with its eyes closed in one corner of the recording chamber. This constituted the behavioral position for QS. During this stage the animal's extremities were relaxed, its shell rested on the cage floor, its eyes were closed and ocular movements were abolished. The neck was extended, the head rested on the plastron or the floor. Heart rate was reduced to a minimum ($\bar{X}$ = 11 ± 3 beats/min), it was statistically significantly lower compared with waking ($p < 0.001$). EEG frequencies slightly declined and amplitudes decreased with sleep. Arrhythmic and intermittent spikes (50–125 μv) occurred monophasically or polyphasically in both cerebral hemispheres (fig. 2A). These electrophysiological signs reached peak levels during behavioral sleep and vanished or declined upon waking. After extended periods of behavioral sleep, short phases of active sleep or REM sleep were observed (fig. 2B). These phases had a mean duration of 19.2 ± 8.5 s. They were accompanied by motor automatisms which consisted of limbs and neck stretches, jaw and ocular movements (single or conjugated). The animals continued sleeping throughout these automatisms. During this sleep phase heart rate was significantly faster than that observed during QS (mean 21 ± 5, $p < 0.005$).

Stimulus presentations during AW and QW elicited head and limbs withdrawal and shell closure. Subjectively it was noted that when the animal was in QS, there was a higher threshold for response to stimuli.

In fact, no clear distribution of the vigilance states throughout the day was seen, but in two animals a slight increase in PS frequencies was observed between midnight and 06.00 h; however, this increase was not significant.

Discussion. It is always precarious to compare the behavior of species phylogenetically distant from one another; similar behavior may actually serve different functions in different species. As we do not yet understand the functional significance of sleep, we remain fixed essentially at a behavioral level. Proceeding at this level, there is no question that behavior observed in *Kinosternon* sp. bears a definite resemblance to what is called sleep in mammals and birds: total immobility, a characteristic posture, continuous eyelid closure, diminished responsiveness to stimulation, etc.

On the other hand, it has been suggested that mammalian slow wave sleep and reptilian spikes are functionally similar[1]. In mammals the elaboration of slow waves and spindles during sleep depends on the integrity of the neocortex[9]. Since reptiles have only a rudimentary cortex[10] it is unlikely that the electrophysiological expression of the spikes during behavioral sleep depends on the neural activity of this thin cellular layer. Furthermore, the thalamo-cortical system responsible for EEG rhythmicity is poorly developed in reptiles[11]. However, studies in the cat have shown that spikes recorded from the ventral hippocampus during SWS are similar to spikes recorded from the reptilian brain during behavioral sleep[12].

When studying the states of vigilance, motor automatisms may be observed during the sleep of poikilotherm vertebrates, such as fish[13], amphibians[14], and reptiles[2,4,15]. These automatisms, also observed in *Kinosternon* sp., were accompanied by ocular movements. A similar behavior is displayed by birds and mammals during typical states of REM or paradoxical sleep. REM sleep has been reported in the chameleon, since ocular movements, either isolated or grouped, were observed during behavioral quiescence[16]. Moreover, this sleep phase has been reported also in the iguanids *Ctenosaura pectinata*[5] and *Ctenosaura similis*[4] as well as in the desert iguana *Dipsosaurus dorsalis*[17]. From the present facts, it seems plausible to conclude that the tortoise *Kinosternon* sp. displays behavioral characteristics of REM sleep.

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Carnosine-like immunoreactivity in the primary olfactory neuron of the rat[1]

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Summary. Using the peroxidase-antiperoxidase immunohistochemical technique, carnosine-like immunoreactivity was demonstrated to localize specifically within the primary olfactory neuron.
Key words. Carnosine; neurotransmitter; olfactory bulb; primary olfactory neuron; immunohistochemistry.

Carnosine (β-alanyl-L-histidine) was shown biochemically to be present within the primary olfactory neuron[2,3]. The concentration of this dipeptide in the olfactory epithelium and olfactory bulb was 10–20-fold higher than that in any other region of the

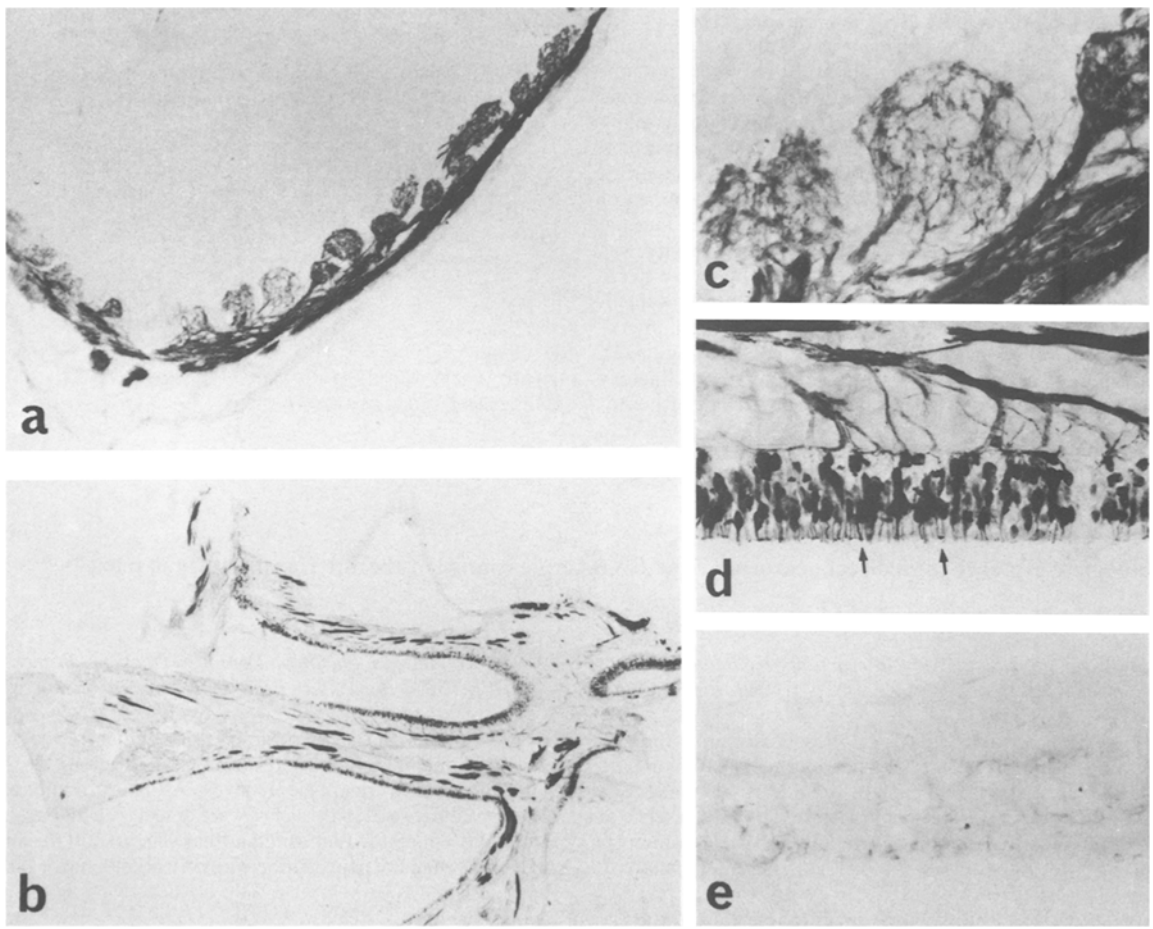
forebrain[3,4]. Biochemical studies have shown that carnosine is synthesized within the olfactory epithelial region, transported, and released from the terminals in the glomerular layer[2-6].

The release of carnosine from glomerular synaptosomes was also observed to be stimulated by depolarizing potassium concentrations and was calcium-dependent[8]. Electrophysiological studies using the extracellular recording technique showed that carnosine displayed an excitatory effect on the mitral and tufted cells, and that this action was accompanied by a secondary increase in granule cell inhibitory activity[9]. These results support the theory that carnosine may be a neurotransmitter in this peripheral input. In the present study, we used an antibody to carnosine conjugated to bovine serum albumine (BSA) to demonstrate the localization of this dipeptide in the primary afferents of the olfactory system in the rat.

Materials and methods. Preparation of antiserum: 60 mg of BSA was dissolved in the 20 ml of 0.05 M phosphate buffer (PB; pH 7.2) and added 113.1 mg of carnosine (0.5 mmoles). During stirring 0.1 ml of 50% glutaraldehyde (GA) was added dropwise and mixed for 15 h at room temperature. This aliquot was dialyzed against 0.005 M PB for 2 days at 4 °C. This carnosine-BSA (protein concentration 4.06 mg/ml) was emulsified with Freund's complete (first time only) or incomplete adjuvant, and injected s.c. in the backs of rabbits biweekly at a dose of 0.5–0.8 mg of the conjugate. The procedure was based on the newly introduced methods which produce antisera against small molecules[10,11]. The antiserum was obtained after 2–3 months immunization. This serum was first purified by precipitation of non-specific BSA antibody, and crude IgG fraction was further obtained by precipitation with ammonium sulfate at a final concentration of 33%.

Preparation of tissues: Three male Wistar rats (200–250 g) were anesthetized and perfused through the ascending aorta with 50 ml lactate Ringer, and subsequently with 400 ml of 4% paraformaldehyde in 0.1 M PB (pH 7.2). The brains and nasal cavities were removed and placed in the same fixative for 2 days. After rinsing 2–3 days in PB with 5–20% sucrose, the olfactory bulbs were cut into 20-μm thick sections frontally, and nasal cavities were cut into sections of the same thickness sagitally on a cryostat. These sections were floated in 0.01 M phosphate buffered saline (PBS) with 0.3% Triton X-100 for at least 1 week, and then processed according to the peroxidase-antiperoxidase (PAP) immunohistochemical technique. Antiserum to carnosine (IgG 3.0 mg/ml) was diluted 1/500–1/2000 and used for incubation at 4 °C overnight. Goat anti-rabbit immunoglobulin (Miles) diluted 1/200 and rabbit PAP complex (DAKO) diluted 1/200 were used for staining at room temperature for 1 h each. The detailed procedures have been published elsewhere[12].

Characterization of antiserum: In a control test, absorbed antisera with related substances bound to BSA (carnosine-BSA, homocarnosine (GABA-histidine)-BSA, GABA-BSA, L-histidine-BSA, β-alanine-BSA) were used for the immunohistoche-



Photomicrographs of carnosine-like immunoreactivity. *a* Olfactory bulb, frontal section. (×20). Olfactory nerve layer and glomerular layer show immunoreactivity. *b* Nasal cavity, sagittal section. (×8). Olfactory epithelium and axon bundle show immunoreactivity. *c* Glomerular layer in olfactory bulb. (×100). Carnosine-positive nerve terminals make glomeruli in glomerular layer. *d* Olfactory epithelium and axon bundles. (×100). All bipolar cells and their axon bundles show positive reactions. Dendrites and their terminal swellings are clearly seen (arrows). *e* The section of olfactory epithelium preabsorbed with antigen (carnosine-BSA). (×100). No reactivity can be seen in bipolar cells.

mical staining, instead of primary antiserum. These conjugates were added to the diluted antiserum (1/500) at a molar ratio of 2:1. In sections, absorption with homocarnosine-BSA as well as carnosine-BSA abolished the immunostaining (fig., e), but addition of other conjugates (GABA-BSA, L-histidine-BSA, β-alanine-BSA) did not affect the staining. For further analysis of cross reactivity, an enzyme-linked immunosorbent assay (ELISA) was performed. This ELISA method was based on our previous report[13]. First, 50 µl of poly-L-lysine solution (1 mg/ml) was dropped into each well of a microtest plate (FALCON 3072) left for 12 h, rinsed with PBS-Tween, and activated by adding 100 µl of 10% GA for 1 h. After rinsing with PBS-Tween, 10 µl of each amino acid solution (1 mM) was placed in the well and left for 12 h. Carnosine (Carn), homocarnosine (h-Carn), GABA, L-histidine (His), β-alanine (β-Ala), glutamate (Glu), aspartate (Asp), glycine (Gly), taurine (Tau), glutamine (Gln), proline (Pro), and cystein (Cys) were prepared as antigens. After blocking free aldehyde with L-lysine (1 M), and preincubation with 200 µl of 2% gelatin, the PAP immunohistochemical method was applied to detect the presence of amino acid-GA-polylysine complexes (coloring with o-phenylenediamine). Cross-reactivities towards these amino acids were obtained by reading the absorbance (OD 490 nm) of each well in the auto-analyzer (Immunoreader, InterMed). The cross-reactivity of this antiserum towards h-Carn was 84%. No cross-reaction was detectable with other amino acids (GABA, His, β-Ala, Glu, Asp, Gly, Tau, Gln, Pro and Cys).

Results and discussion. Immunohistochemical investigation using the antiserum to carnosine (IgG, 3.0 mg/ml, 1/1000–1/2000) revealed that the primary olfactory neuron was specifically labeled, but no labeled neuronal element was found in the remaining regions of the brain. This immunoreactivity was not observed after incubation with antiserum preabsorbed with antigen (carnosine-BSA) (fig., e). In the olfactory epithelium, many olfactory cells of bipolar shape and fiber bundles also showed a positive reaction (fig., b, d). Strongly labeled apical dendrites and terminal swelling of dendrites were noticed in the lumen of the nasal cavity, and basal thin axons could be followed into the axonal bundles. In the olfactory bulb, immunoreactivity was restricted exclusively to areas within both the olfactory nerve and glomerular layer, but no neuronal perikaryon was stained. Dot-like axon terminals demarcated the profiles of the glomerulus, where numerous labeled nerve fibers terminated (fig., a, c). Concentration of carnosine in the olfactory nerve or olfactory bulb was reported to be 50–100-fold as much as that of homocarnosine by a biochemical assay[4]. This suggest that immunoreaction detected in the present investigation is mostly due to carnosine, though our antiserum does not differentiate carnosine from homocarnosine. A selective decrease (5–15%) in the carnosine level in peripherally deafferentiated olfactory bulb[4] also supports our result. It should also be noted that dendrites toward the olfactory epithelium and their terminal swellings have such an amount of carnosine (fig., d). Carnosine may have some function in this region.

The present immunohistochemical study shows that carnosine-like immunoreactivity is specifically localized within the primary olfactory nerve. This evidence strongly supports the hypothesis that carnosine is the neurotransmitter in the primary olfactory neuron.

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Morphological evidence for a direct neuroendocrine GABAergic control of the anterior pituitary in teleosts

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Summary. The anterior pituitary of teleosts is unique among vertebrates in receiving a direct innervation which represents the morphological support of the neuroendocrine control of pars distalis functions. The participation of GABAergic fibers in this innervation was studied by means of immunocytochemistry at the light and electron microscopic levels, using antibodies against GABA. Immunoreactive fibers, characterized by the presence of small clear and dense cored vesicles, were detected in all parts of the gland. Immunopositive terminals were found in close, sometimes synaptic-like, contact with most glandular cell types in the anterior lobe. The data strongly suggest that in teleosts, as in mammals, GABA is involved in the neuroendocrine control of anterior pituitary functions.

Key words. GABA; immunocytochemistry; anterior pituitary; neuroendocrine control; teleost.

GABA is classically considered to represent one of the major inhibitory neurotransmitters in the central nervous system. Furthermore, a growing body of evidence obtained from mammals suggests that GABA may also be involved in the neuroendocrine regulation of adenohypophyseal functions[1–4]. The suggestion that GABA plays a role in the release of MSH[5,6] is supported by the fact that GABAergic fibers have been detected in the intermediate lobe[7–9]. Because of the absence of nerve fibers in the