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The Threshold Sensitivity of External Chemoreceptor in Carp *Cyprinus carpio* to Amino Acids and Classical Gustatory Substances

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Abstract—The chemical sensitivity of five local sections of carp *Cyprinus carpio* were studied on the anterior part of the head, by recording the slow electric potentials from the skin's surface, in response to L-amino acid (cysteine, histidine, phenylalanine, and proline) solutions and the solutions of classic gustatory substances (quinine-HCl, quinidine-H_2SO_4 (D-quinine), sodium chloride, citric acid, and saccharose). The stimuli caused changes in the concentration-dependent potential. It was found that the surface around the base of large barbel possessed maximum sensitivity to chemical stimuli. Cysteine was the most efficient substance in all tap points. The results of receptor zone rank, according to threshold sensitivity to cysteine, was the base of the large barbel 6.5×10^{-6} > gular region 3.7×10^{-5} > center of the upper lip 5.0×10^{-5} > suborbital region 8.0×10^{-4} > interorbital space 3.0×10^{-3}. Saccharose possessed the minimum stimulatory efficiency with an average threshold concentration of 10^{-2} to 10^{-1} M, depending on the zone of measurement. The change of solution pH within the range 5.0–9.0 did not affect the level of sensitivity. The average threshold values are assumed to reflect the density of taste buds in the recorded sites.

The sensory systems receiving chemical stimuli in fish, like other water inhabitants, play a significant role in the intrapopulational and interspecies relationships with hydrobionts in orientation, migration, foraging, defense, and other forms of fish behavior. The importance of chemical channels of information in the life of a fish is indicated by the fact that fish have four different sensory systems, each receptive to chemical signals: the olfactory system, the gustatory system (with two subsystems, the intraoral and the external), solitary chemical cells, and the common chemical sense which has been least investigated and does not have a clear definition (Finger, 1997). Every system has its own receptors. The olfactory system is represented in the peripheral part with specialized primarily sensing olfactory cells situated in the paired olfactory organs (Bronshtein, 1950). The secondary-sensing taste cells appear in the receptor apparatus of the gustatory system (intraoral and external), which together with other supporting cells forms taste buds, localized in the epithelium of the mouth, pharynx, branchial cavity, and all over the surface of the fish body (Reutter, 1986; Gomahr *et al.*, 1992; Finger, 1997). Solitary chemical cells, also secondary-sensing, are distributed on the surface of the body and fins in cyclostomata, chondrosteans, and teleosts (Whitear, 1971b; Kotrschal, 1991, 1992; Peters *et al.*, 1991, Whitear, 1992). It is generally agreed that free sensory nerve endings, which are prevalent in the epidermis of fish (Whitear, 1971b, 1983), are the morphological substrate of common chemical feeling (Parker, 1912; Silver, 1987).

The dermal surface of a fish's body includes three types of chemosensory structures: taste buds, solitary chemical cells, and free nerve endings of cranial (on the head) and spinal (on the trunk) nerves. The behavioral responses in water environments to dissolved chemical substances probably occurs with the participation of these chemoreceptor structures (Devitsina, 2003). As long as their density significantly differs, depending on their localization on the surface of the fish (Whitear, 1971a, 1971b; Gomahr *et al.*, 1992; Kotrschal, 1992), the development of mapping the body surface of fish, according the criterion of integrated sensitivity to different types of substances, is of interest.

This aim of this study was to investigate the integrated responses and thresholds to the solutions of chemical substances, by recording the electric potentials in localized parts of the skin surface in carp *Cyprinus carpio.*

MATERIAL AND METHODS

The experiments were carried out in the fall and winter on 2-year-old carps, 14–17 cm in length, which were kept in a laboratory aquarium for no less than 6 months and fed with living food (midge). A total of 47 carps were used. Before the experiment, the fish were wrapped up with a moist cotton cloth and placed in a foam socket, and fixed in a box with running water.

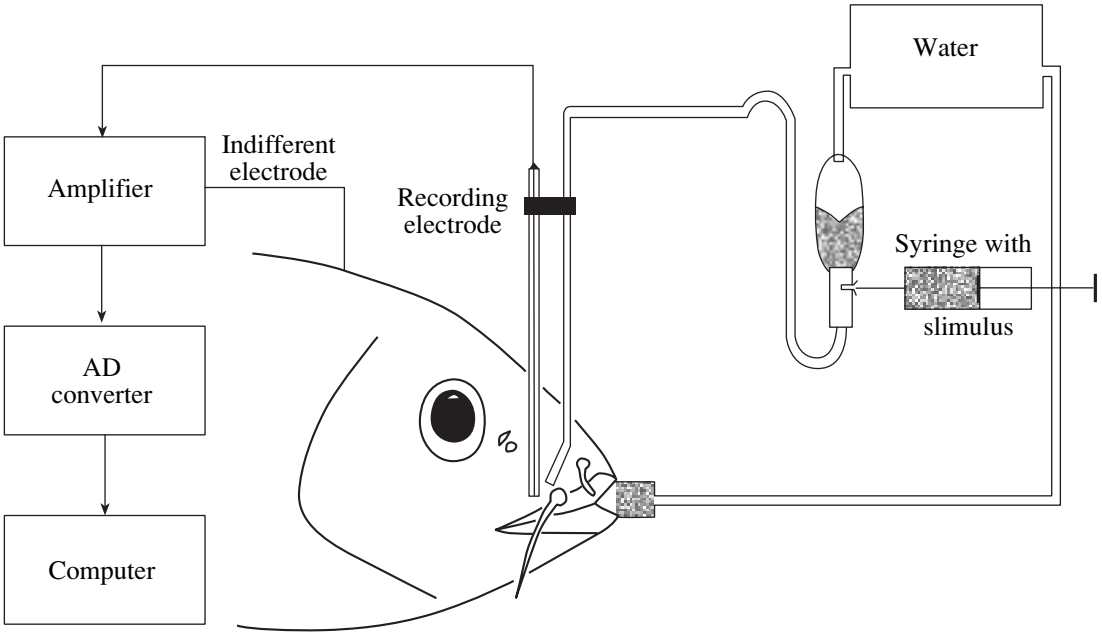

Fig. 1. A diagram of the electrophysiological device used for recording the responses of skin chemoreceptors.

Water came continuously from the sedimentation tank, perfusing the gills at a rate of 150 ml/min. Water temperature was 18–20°C. Slow-electric potentials were recorded, from the skin's surface, in the anterior part of the head, in response to local imput of stimulus. Myorelaxants and anesthetics were not used. The fish were returned to the aquarium, after the experiments, to swim and feed normally.

Stimulation procedure. A local section of skin surrounding the recording electrode was sprayed with a small water jet from the sediment tank, which flowed from a glass capillary (inner diameter 1.0–1.2 mm) at a rate of 25 ml/min. A stimulus solution (2 ml) was added to this flow with a syringe, separate for every substance, at a 2-s period. To damp out a pressure shock during the injection of a stimulus, an air reservoir was included in the channel system (Fig. 1).

We carried out a series of measurements of the absorption spectra of phenylalanine solution before and after it passed through the stimulating channel system. This determined how much the stimulating solution concentration decreased, when mixed with water, at the moment of its contact with the skin's surface, and compared it with the result of the initial solution. The absorption spectra were estimated in the band of 190–350 nm in Hitachi-557 spectrophotometer (Japan) (model experiment 1).

Chemical stimuli. Solutions of 10^{-8}–10^{-2} M L-amino acids, cysteine, histidine, phenylalanine, and proline, and solutions of classic gustatory substances, quinine chloride and quinidine sulfate (D-quinine) 5×10^{-6}–5×10^{-3} M, sodium chloride and citric acid 10^{-5}–5×10^{-3} M, and saccharose 5×10^{-3}–5×10^{-1} M (Fluka, Reanal), were prepared with the same settled potable water for both the test stimuli, with which the head and gills were sprayed, as well as the control. The interval between separate offerings of stimuli was 5 min. The order in which the substances were offered arbitrarily varied from experiment to experiment. The temperature of the water and the stimulating solutions was similar. The pH values of different solutions varied from 7.1 to 8.1.

Recording of electric responses. A silver chlorinated wire Ag–AgCl, 25 mm in length and 0.3 mm in diameter, was used as a recording electrode. The electrode was mounted in a glass capillary with an inner diameter of 1 mm. A gap was between the wire electrode and the glass filled with fresh water. The lower end of the capillary, with a preliminarily melted circular edge, was closely pressed against the tested section of skin. The recording started 60 min after the mounting of the electrode. The electric signal from the electrode, after its amplification and low-frequency filtration (constantly every 0.2 s), entered the analog digital converter (ADC), where it was converted to a digital form, through the parallel input/output port to the computer memory. The quantization period of the source of the ADC signal was 10 ms. The balancing of the amplifier (setting the output to zero) was performed automatically, under the control of the author's program, in the intervals between the recordings of electrophysiological responses. Fluctuations of electric potential reflected the overall responses of dermal chemoreceptors. The amplitude, duration, and lag phase of a response were seen on a monitor screen.

The concentration of stimulus was considered as its threshold when the amplitude of the electric response

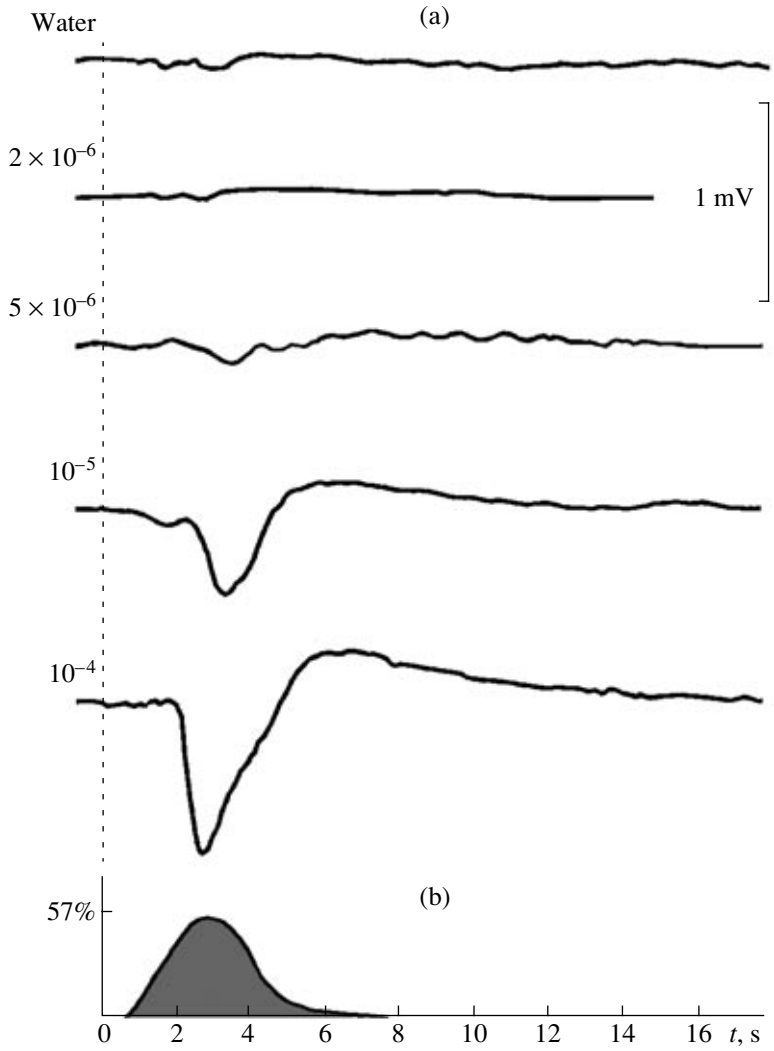

Fig. 2. Responses of skin chemoreceptors in the gular region of carp *Cyprinus carpio* to cysteine (M) solutions (a) and the change in concentration of the tested substance in the channel in the process of stimulation (b). The concentration of initial solution is taken at 100% concentration.

was two times greater than the possible deviations of the potential, against a background of offering the control stimulus of pure water. After the threshold was determined, additionally electric responses were recorded in the same tap point within the threshold concentration of 0.5- to 10-fold. The response thresholds for several substances were studied on one specimen, in several points of head surface (six tests of one substance per every point). The conclusions made about the significance of the differences between the recorded potentials, before the stimulation and in response were verified by the Wilcoxon test, at the significance level $p < 0.05$.

The subsequent processing of the data included calculations of the averaged logarithmic values of threshold concentrations (measured the effect of the same type of substance on different individuals, $n = 6$), which were then transferred to the decimal-power form of presentation of the solution concentrations.

RESULTS

Skin's electric responses to chemical stimulation. As can be seen from Fig. 2a, the recorded electrophysiological responses to water were close to the level of background activity. The introduction of stimulating solutions to the channel caused the concentration-dependent potential changes to increase during the time of induction for 1–2 s and then a subsequent decrease back to the initial level. Usually the response lasted for 3–5 s. The response of amplitude varied between 0.2 to 1.5 mV depending on substance type and concentration. A curve shift to the left was observed when stimulus concentration increased. A small potential spike with a short time lag (in comparison with the response to chemical stimulus) was occasionally observed in the

Average values of threshold concentrations (M) of chemical stimuli for five zones of the head surface in carp *Cyprinus carpio*

Chemical stimuli	Zones				
	Gular region	Base of great maxillary barbel	Center of the upper lip	Suborbital space	Interorbital space
Cysteine	3.7×10^{-5}	6.5×10^{-6}	5.0×10^{-5}	8.0×10^{-4}	3.0×10^{-3}
Phenylalanine	5.9×10^{-5}	1.1×10^{-5}	2.0×10^{-5}	3.2×10^{-3}	5.0×10^{-3}
Histidine	3.2×10^{-4}	2.0×10^{-5}	3.2×10^{-5}	5.0×10^{-4}	5.0×10^{-3}
Proline	10^{-4}	4.0×10^{-5}	5.0×10^{-5}	1.6×10^{-2}	3.2×10^{-2}
Quinine chloride	10^{-4}	8.5×10^{-5}	5.0×10^{-5}	5.0×10^{-4}	10^{-3}
Quinidine sulfate	3.2×10^{-4}	2.0×10^{-4}	2.0×10^{-4}	1.6×10^{-2}	3.2×10^{-2}
Citric acid	2.0×10^{-4}	2.0×10^{-4}	2.0×10^{-4}	5.0×10^{-4}	5.0×10^{-4}
Sodium chloride	7.9×10^{-4}	10^{-3}	5.0×10^{-4}	10^{-3}	6.3×10^{-4}
Saccharose	3.3×10^{-2}	1.6×10^{-2}	10^{-2}	10^{-1}	10^{-1}

initial stages of response to water or stimulus solution (Fig. 2a).

Sensitivity thresholds of skin chemoreceptors in carp. The sensitivity thresholds to four amino acids and four classical gustatory substances were determined in five zones (see table). The experiments demonstrated that the sensitivity thresholds to the substances differed 2–3 times in the different zones of the carp's head. As seen in the table, the maximal sensitivity to chemical stimuli was recorded at the base of the great barbel and in the center of the upper lip. In separate records, the threshold concentrations here reached 10^{-7} M under the effect of cysteine, which was the most efficient stimulus in all tap points. The zones studied are arranged in the following series according to their threshold sensitivity to cysteine: the base of great barbel, gular region, center of the upper lip, suborbital region, interorbital space. Saccharose had the least stimulating efficiency.

Model experiments were carried out in order to determine the actual concentrations of stimulus solutions near the zone of electrode contact, with the surface of the fish's skin and the effect of solution pH on the values of the measured thresholds.

Determination the concentration of stimulus solution. Two milliliters of phenylalanine solution, 10^{-3} M in concentration were administered to the flow, and separate test tubes were filled by pouring out liquid from capillary every s. Then, the optical density of these solutions was measured and the concentration of phenylalanine was calculated in each tube. It was determined that the amino acid solution mixed with water and poured out from the capillary for 6 s, from the second to the seventh s starting from the moment of introduction (Fig. 2b). A concentration peak was observed during the third and the forth s (from the moment of the introduction of a stimulus solution to the system); the concentration of the stimulus substance in the water, pouring out of the capillary, comprised about 57% of the initial level in this 2-s period (Fig. 2b). This data was then used to calculate the actual threshold concentrations

Effect of solution pH. The effect of the pH of tested solutions on the electrophysiological responses of chemoreceptors was studied in two series of experiments. In the first experiment, amino acid cysteine solutions were tested (in concentrations of 10^{-7}–10^{-3} M) as well as, quinine chloride (5×10^{-4}–5×10^{-3} M), pH of which was adjusted with 0.1 N NaOH to the pH of aquarium water 7.9. It was determined that the threshold at this pH averaged in the base of the great barbel region 10^{-6} M ($p < 0.05$, $n = 6$) for cysteine and 5×10^{-4} M ($p < 0.05$, $n = 6$) for quinine.

The responses of chemoreceptors in the same zone, to a concentration of 10^{-5} M cysteine solution, at pH values 5.0, 6.0, 7.0, 8.0, and 9.0, were studied in the other series of experiments. It was found that the pH in this range does not affect the value of the response; the same picture was observed for a 5×10^{-4} M concentration of quinine solution.

DISCUSSION

Quick recognition of signal substances present in the water is necessary for fish to adapt efficiently to their environment. Under natural conditions, an animal makes a decision and correspondingly performs behavior acts, for example, to search for food, on the basis of this complex information received from the many sensory systems (Pavlov and Kasumyan, 1990). The external chemosensory systems play an important part in this. The external taste buds and solitary chemical cells (SCC), together with olfaction, play the part of distant analyzers, which are responsible for the feeding behavior, as well as for the detection of the sources of hazard signals (Atema, 1971; Gomahr *et al.*, 1992; Whitear, 1992).

Carp is a benthos feeder with a demersal mode of life. It feeds on animal and plant food. Organs of smell, touch, and taste play the main role in its search for food (Nikolskii, 1974). The gustatory system of Cyprinidae

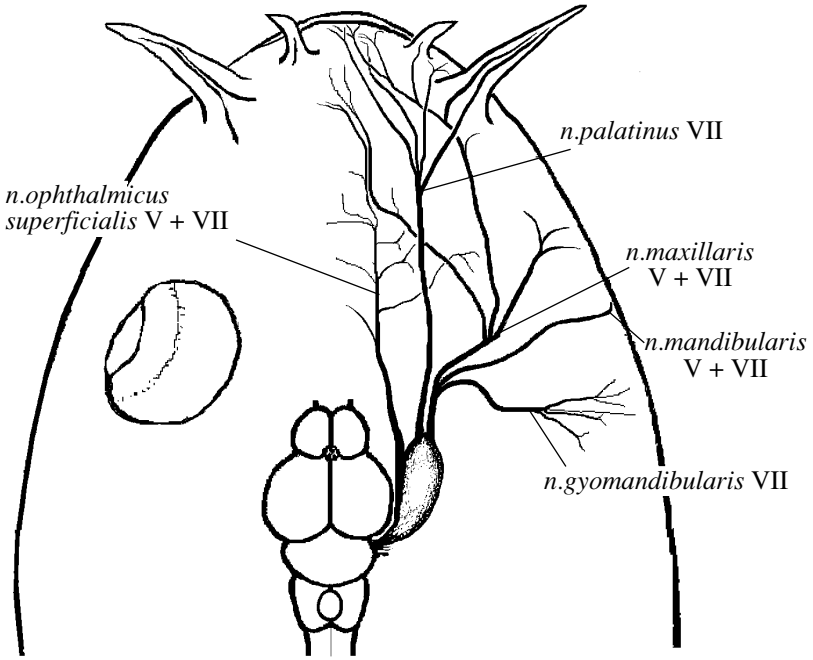

Fig. 3. A diagram of innervation in the front part of the head of carp *Cyprinus carpio* with the branches of *n. trigeminus* (V) and *n. facialis* (VII).

is highly developed and is represented by multiple intramouth and external taste buds covered irregularly across the surface of its body (Marui and Caprio, 1992).

A previous study about ten species of carp fish (carp was not included in their number) demonstrated that bottom-dwelling fish fed on benthos and have greater number of external taste buds than those who live in open water or near the water's surface. The density of taste buds decreased, in most studied species, from the rostral to the caudal and from the ventral to the dorsal surfaces (Gomahr *et al.*, 1992). Similar to carps, the maximum density of external taste buds in bullheads Ictaluridae and codfishes Gadidae was found in those areas which come in contact with food most often: on the lips, barbels (if they are presented), around the orbits, on opercles, in the gular region, on pectoral and ventral fins (Atema, 1971; Bardach and Atema, 1971; Kiyohara *et al.*, 1980; Caprio, 1988; Jakubowski and Whitear, 1990; Gomahr, *et al.*, 1992).

In our experiments, the chemosensitivity of different parts of the skin in carps differed significantly. The lowest thresholds were recorded at the base of great maxillary barbel, and the highest thresholds in the interorbital and suborbital spaces (see table). This data correlates with morphological data on high concentration of taste buds on the upper lip and barbels of carp (Bardach and Atema, 1971), which in turn correlates with morphological data on innervation of the frontal part of the head in carp and other fish in the *n. facialis* (VII) and *n. trigeminus* (V) branches carrying information about chemical and tactile stimuli (Luiten, 1975; Marui and Funakoshi, 1979; Chervova and Devitsina, 1981; Devitsina and Chervova 1983; Bartheld and Meyer, 1985; Puzdrowski, 1988; Kiyohara *et al.*, 1999). Our observations and available literature data (Kiyohara *et al.*, 1985) suggest that great maxillary barbel is innervated with very large offsets of two nerves, *n. palatinus* VII and *n. maxillares* V + VII (Fig. 3). Multiple thin bundles of these nerves branch out in the region of the upper lip. Small bundles of *n. ophthalmicus superficialis* V + VII and *n. buccalis* V + VII innervate the interorbital and suborbital spaces, respectively. The gular region and lower lip are innervated with strong *n. mandibularis* V + VII. The presence of tactile fibers in the nerve bundles determine the integration of information on chemical quality with tactile information about the food (Funacoshi *et al.*, 1981; Davenport and Caprio, 1982; Ogava *et al.*, 1997). Some taste buds rise above the surface of epithelium to ease the perception of tactile stimuli (Devitsina, 2003). Due to close spatial and functional combining of tactile and chemoreceptor systems, the recording of responses to chemical stimuli from the skin's surface could be difficult as a result of the electrical summation of chemical and tactile components. We sometimes observed a small potential spike, at the beginning of stimulation, which was presumably connected with the activation of tactile receptors. However, tactile components in most cases were not pronounced due to our system of stimulus solution supply (Fig. 2a).

Our experiments demonstrated that amino acids and classical gustatory substances (excluding saccharose)

efficiently stimulated the external chemoreceptors of carp (see table). A high sensitivity to amino acids was observed in the other fish species, when recording the activity of facial and trigeminal nerve trunks innervating the surface of the body and frontal part of the head (Funakoshi *et al.*, 1981; Davenport and Caprio, 1982; Belousova *et al.*, 1983; Marui *et al.*, 1983; Chervova *et al.*, 1985, 1989; Caprio, 1988; Marui and Caprio, 1992). The electrophysiological thresholds that were determined by recording of the activity of the facial nerve, for the most efficient amino acids, in most of the species of teleost fishes studied, varied from 10^{-6} to 10^{-9} M (Marui and Caprio, 1992). It was shown that the most efficient amino acids were different for different species.

In our experiments, amino acid cysteine possessed maximum efficiency, and to a lesser degree phenylalanine, proline, and histidine. This data agrees with the results of carp behavioral experiments, according to which amino acids used in our experiments belong to three different groups categorized by their gustatory attractability to carp. Cysteine and proline are highly attractive, while phenylalanine is a deterrent, possessing a repulsive taste, and histidine is neutral (Kasumyan and Morsi, 1996; Kasumyan and Døving, 2003). In the electrophysiological experiments on carp, when tapping from *n. mandibularis* VII innervating the lower jaw, cysteine was third in its efficiency after proline and alanine of the 30 tested amino acids, (Marui *et al.*, 1983).

The thresholds for classic gustatory substances obtained in our experiments are similar to the thresholds of substances calculated by the records of activity from the facial nerve of carps (Funakoshi *et al.,* 1981). A low sensitivity to saccharose by external chemoreceptors observed in our experiments (10^{-2}–10^{-1} M) is in agreement with available literature data (Bardach and Case, 1965; Funakoshi *et al.*, 1981; Kasumyan and Morsi, 1996). All this suggests that the recorded responses were mediated with external taste receptors.

The adding of stimuli to the channel could, for a short time, change the environmental pH near the studied skin area. These changes in turn could modify the responses of chemoreceptors or cause a potential shift on the electrode connected with the receptor activity. Our experiments demonstrated that solution pH in the range from 5.0 to 9.0 did not affect the chemoreceptor's responses recorded from the skin surface of the fish. Agreeing with the published data, the responses of neurons in the facial part of the carp did not depend on the pH level of the solution sprinkled on the surface of the skin around the mouth (Vasilevskaya and Polyakova, 1977). The responses of gustatory chemoreceptors obtained in eels *Anguilla anguilla* and rainbow trout *Oncorhynchus mykiss* were to pH above 8.0 (Yoshii *et al.*, 1980; Yamashita *et al.*, 1989), while in carp they were to pH below 6.0 (Marui *et al.*, 1983). Hence, in our experiments, we can interpret all the changes of skin potentials as the responses of chemoreceptors to adequate chemical substance stimulation.

Solitary chemical cells (SCC) are abundantly present in the epidermis of fish, along with taste buds. They are distributed on the surface of the body more uniformly than taste buds (Kotrschal, 1991, 1992; Whitear, 1992). The SCC in the first dorsal fin of rocklings *Ciliata mustela* and *Gaidropsarus mediterraneus* having a great density, are highly sensitive to the components of skin mucus of possible predators and can mediate, hence a defensive behavior (Peters *et al.*, 1987, 1991). In the free rays of the pectoral fins in *Prionotus carolinus*, SCC are also abundant, as they are innervated with spinal nerves. For the same purpose that *Prionotus carolinus* uses SCC to search for food, other fish use a system of external taste buds (Finger, 2000). This study demonstrated that the chemoreceptors in the fin rays of Gurnards respond to food extracts, amino acids (cysteine, proline, phenylalanine, etc.), and classical gustatory substances, excluding saccharose (Bardach and Case, 1965; Bardach *et al.*, 1967; Silver and Finger, 1984).

Additionally, the epidermis of fish is penetrated with free nerve endings, which can be under or between the surface of the epithelial cells (Whitear, 1971a, 1983). It is assumed that the fastest components of the chemosensory responses belong to the activity of the free nerve endings surrounding the taste buds (Bardach and Case, 1965).

It's likely that all surface chemosensory structures (taste buds, SCC, and free nerve endings) take part in the formation of electric responses recorded in carp to chemical signals. Contributing and complementing each other, they obtain the most complete characteristics of the chemical signals. The information that they sent to the different parts of the central structure promotes the formation of adequate behavioral responses. However, the main component of response is, undoubtedly, provided by the external gustatory reception.

Our method of determining the threshold sensitivity allowed us to measure the integrated responses of all the chemoreceptors present in the skin, hence making up a representation of their density in the different parts of skin surface. The maximum electrical activity of the external chemoreceptors of Carp were recorded around the great maxillary barbel, on the upper lip, and in the gular region, thus allowing us to assume a comparatively great density of the chemoreceptors in these areas belonging, to the external taste system.

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