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Circular Swimming by the Medaka, *Oryzias latipes*, Induced by Microinjection of GABA-ergic Agonists and Antagonists into the Posterior Thalamus

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Abstract The thalamic mechanism involved in locomotor control, particularly in steering control, was studied in a teleost, *Oryzias latipes*. Ipsilateral circular swimming was induced by injection of bicuculline, a selective gamma-aminobutyric acid (GABA)-A antagonist, into the unilateral posterior thalamus. δ-Amino-valeric acid, phaclofen and 2-hydroxysaclofen (weak GABA-B antagonists) had weak, but similar effects. In contrast to antagonists, injection of GABA, muscimol (GABA-A agonist), baclofen (GABA-B agonist), nipecotic acid (blocker of Na^+-coupled GABA uptake carrier), and γ-hydroxybutyric acid (possible GABA-A and -B agonist) into the unilateral posterior thalamus induced contralateral circular swimming. It is suggested that GABA-ergic inhibition in the posterior thalamus, probably via both GABA-A and -B type receptors, plays an important role in the steering control of teleosts when swimming.

In vertebrates, including lower vertebrates such as teleosts, directional control during locomotion is by multiple neural centers in the brain, namely, basal ganglia (Iwamoto and Way, 1977; Oberlander et al., 1977; Olpe et al., 1977; Dewar et al., 1983), the thalamus (Di Chiara et al., 1979), the mesencephalic superior colliculus (or optic tectum) (Meyer et al., 1970; Imperato and Di Chiara, 1981; Di Chiara et al., 1982) and the reticular formation (Mulas et al., 1981). Electrical stimulation of the optic tectum in trout (Akert, 1949), for example, induced movements of the eyes, head and body in a definite direction, such movements being thought comparable to the visual grasp reflex (Hess et al., 1946). Similar electrical stimulation in freely swimming cod caused circular swimming (Manegebewegungen) (Meyer et al., 1970), which appears to be one of the complex of reactions associated with the visual grasp reflex.

On the other hand, various neurotransmitters have also been reported as been involved in the activation and/or modulation of the motor outputs. Grillner et al. (1991) showed the important role of excitatory amino acid during fictive locomotion in the lamprey spinal cord. GABA-A and GABA-B-mediated effects influenced intersegmental coordination; that is, the GABA system was active during normal locomotor activity in the lamprey (Tegner et al. 1993). In mammalian spinal cord in vitro preparations, activation of glutaminergic and serotonergic receptors have been shown to induce locomotor activities (Cazalets et al., 1990). Cholin-ergic and GABA-ergic neurons were found in the brain (telencephalon, diencephalon, optic tectum and tegmentum) of a teleost (Brantley and Bass, 1988; Martinol et al., 1990) and high concentrations of GABA and glutamate were recently found in the fish brain (Sloley et al., 1992). GABA is most probably one of the major neurotransmitters in the telencephalon and diencephalon of teleosts (Martinol et al., 1990).

The aim of the present study was to identify the neural centers responsible for the regulation of circular swimming in teleosts, and to identify the neurotransmitters concerned. To date, a variety of GABA-ergic drugs (agonists, antagonists and uptake-blockers) have been developed for both GABA-A and -B receptors (Johnston et al., 1972; Krogsgaad-Larsen and Johnston, 1975; Curtis et al., 1979; Larsson et al., 1980; Schwarz et al., 1988; McGeer and McGeer, 1989; Kerr et al., 1990), enabling such a study.

Circular swimming has not been observed following injection of GABA-ergic drugs into the telencephalon and anterior thalamus. Furthermore, those fish treated by injection of the drugs into the rhombencephalon could not swim (Takeuchi, unpubl.), whereas injections into the mesencephalon elicited complicated results, similar to those seen following

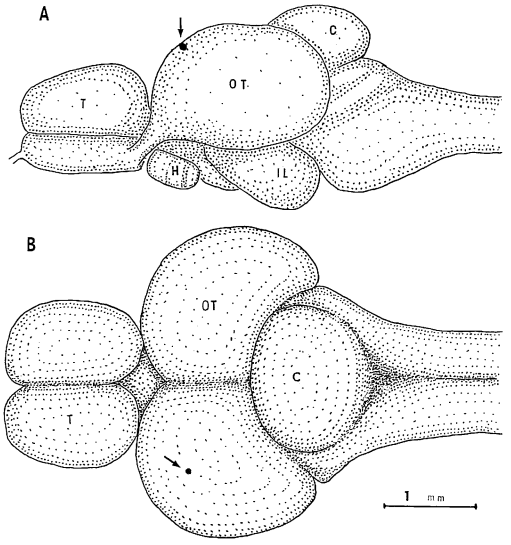

Fig. 1. Brain of medaka. A) Side view; B) top view. *C*—cerebellum; *H*—hypophysis; *IL*—inferior lobe; *OT*—optic tectum; *T*—telencephalon. Arrows: point of injection.

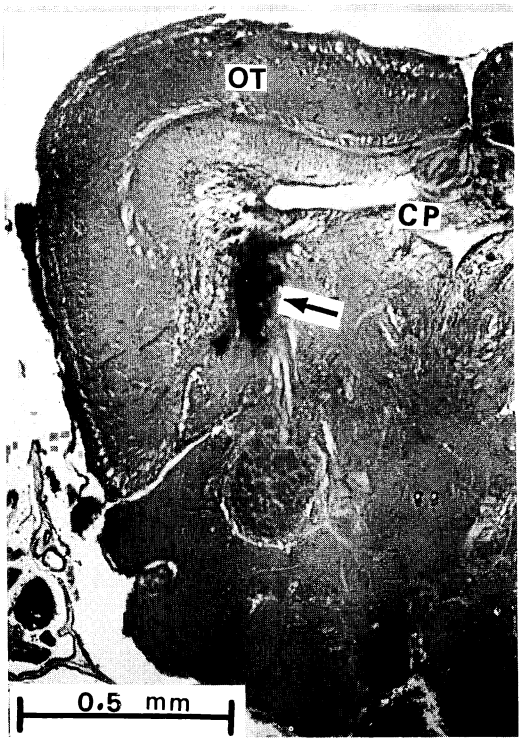

Fig. 2. Left half of cross section at arrows in Figure 1. *CP*—commissura posterior; *OT*—optic tectum; Arrow: injected china ink.

electrical stimulation (Hess et al., 1946; Akert, 1949). Subsequently, the diencephalon (posterior thalamus) was chosen for the present study.

The medaka (*Oryzias latipes*) is common, being found near the surface of small rivers in Japan. It is an excellent model animal for the study of behavioral pharmacology, because the small fish (3–4 cm long) is hardy and easy to raise in small glass dishes.

Materials and Methods

Orange-red medakas, *Oryzias latipes*, were kept outdoors. Experimental fish were anesthetized by immersion in 0.02% phenylurethan for several minutes, positioned with pins on paraffin in a dish filled with saline (Yamamoto, 1939), and a small part of the cranium over the optic tectum removed by forceps under microscopic observation. An appropriate volume of saline containing neurochemicals was microinjected into the brain. The operation and microinjection took about 5 minutes, ending before the anesthesia wore off (usually 10 minutes). The small opening on the cranium appeared to hurt the fish little, the wound healing within several days. Following the operation, the fish was put into a glass dish (10 cm deep and 15 cm in diameter) containing saline (25°C), and its behavior observed for 30 minutes following its recovery from anesthesia. All neurochemicals tested, except baclofen, phaclofen and 2-hydroxysaclofen, were dissolved in saline for medaka. Baclofen, phaclofen and 2-hydroxysaclofen were dissolved in 0.02 N HCl-saline. A micromanipulator with a microinjector (IM-4B Narishige Scientific Inc.) was used for the injection. The tip diameter of the glass micropipette was about 10 μm. The solution was injected by pressure, its volume being estimated from the diameter of the drop issuing from the pipette tip. The total volume of the injections was kept constant at around $1\text{–}5\times10^{-7}$ ml. Neurochemicals used were obtained from RBI Research Biochemicals Inc., Massachusetts, USA. Injection into the lateral posterior thalamus was carried out through the optic tectum (Fig. 1A, B). No abnormal swimming occurred in controls injected with the same amount of either saline or 0.02 N HCl-saline. The injection point was determined by injecting china ink using the same procedure into 8 fish, the ink being subsequently found in the expected locus on almost all of the histological sections (Fig. 2). In two of the latter, the ink was found in the

Table 1. Circular swimming elicited by injection of GABA-ergic agents into the unilateral posterior thalamus of the medaka

GABA-ergic chemicals	Concentration of injected solution mM	No. of fish	Normal swimming	Circular swimming	
				Contralateral	Ipsilateral
Antagonists					
Bicuculline methyl bromide	6	18	0	0	18
Bicuculline methyl chloride	6	3	0	0	3
5-Aminovaleric acid HCl	560	12	8	0	4
Phaclofen	*	6	3	0	3**
2-Hydroxysaclofen	*	9	8	0	1**
Agonists					
GABA	80	5	4	1**	0
GABA	600	7	2	5**	0
Baclofen	5	10***	1	6**	0
Muscimol	20	6	3	3	0
γ-Hydroxybutyric acid	8	3	1	2**	0
Nipecotic acid	15	6	4	2**	0

*Saturated solution used; **circular swimming observed only for a few minutes; ***three fish unable to swim.

Table 2. Effects of neurochemical antagonists injected into the unilateral posterior thalamus of the medaka

Chemicals	Concentration of injected solution mM	No. of fish	Normal swimming	Circular swimming	
				Contralateral	Ipsilateral
Adrenergic					
Alprenolol HCl	10	3	3	0	0
Alprenolol HCl	80	3	3	0	0
Propranolol HCl	*	5	5	0	0
Cholinergic					
Methoctramine 4HCl	5	2	2	0	0
Methoctramine 4HCl	35	3	3	0	0
Scopolamine n-butyl bromide	15	5	5	0	0
Scopolamine n-butyl bromide	220	3	3	0	0
Succinylcholine chloride	30	3	3	0	0
Succinylcholine chloride	300	3	3	0	0
Dopaminergic					
Metoclopramide HCl	25	5	5	0	0
Metoclopramide HCl	360	3	3	0	0
Fluphenazine 2HCl	25	2	2	0	0
Fluphenazine 2HCl	200	3	3	0	0
Trifluoperazine 2HCl	12	5	5	0	0
Trifluoperazine 2HCl	120	3	3	0	0
Histaminergic					
Chlorpheniramine maleate	10	2	2	0	0
Chlorpheniramine maleate	150	3	3	0	0
Serotonergic					
Propranolol HCl	*	5	5	0	0
Mianserin	*	3	3	0	0

*Saturated solution used.

region from the posterior thalamus to the anterior tegmentum.

Results and Discussion

A microinjection of bicuculline (selective GABA-A antagonist) (McGeer and McGeer, 1989; Swann et al., 1989) into the unilateral posterior thalamus induced ipsilateral circular swimming (circular swimming toward the direction ipsilateral to the side of the thalamic microinjection) in all 18 fish examined (Table 1). The diameter of the swimming circle was 5–8 cm, the rate of swimming being about 5–12 seconds per circle. Swimming lasted for 5–30 minutes. The effects were apparently short-lived, as the injected fish swam normally after an hour. While a small quantity (1 ng) of bicuculline caused circular swimming, a large quantity (25 ng) of 5-aminovaleric acid (weak GABA-B antagonist) (Schwarz et al., 1988; Kerr et al., 1990) was necessary to induce the same response. Phaclofen and 2-hydroxysaclofen (weak GABA-B antagonist) (Kerr et al., 1990) had similar weak effects. In contrast, GABA, muscimol (GABA-A agonist), baclofen (GABA-B agonist), γ-hydroxybutyric acid (possible GABA-A and -B agonist) and nipecotic acid (blocker of GABA uptake) (Krogsgaard-Larsen and Johnston, 1975; Larsson et al., 1980) induced contralateral circular swimming when injected into the unilateral posterior thalamus (Table 1). However, the effect of these drugs lasted only for a few minutes, with the exception of muscimol, which persisted for more than 10 minutes. These results suggested that endogenously released GABA (either of intrathalamic or extrathalamic origin) is involved in steering control, and that both type-A and -B GABA receptors are involved.

Of the various neurochemicals injected into the unilateral posterior thalamus, only the GABA-ergics caused circular swimming. All of the following chemicals were ineffective; adrenergics: alprenolol and propranolol; cholinergics: methoctramine, scopolamine n-butyl bromide and succinylcholine chloride; dopaminergics: metoclopramide, fluphenazine and trifluoperazine; histaminergic: chlorpheniramine maleate; serotonergics: propranolol, metoclopramide and mianserin (Table 2).

Both the GABA-B antagonists (5-aminovaleric acid, phaclofen, 2-hydroxysaclofen) and the GABA-B agonist (baclofen) effected only a few of the fish treated (Table 1), whereas a low concentration of GABA-A antagonist (bicuculline) affected all of the fish examined. This suggested that activation of the GABA A-receptors is the major factor, the B-receptors playing a minor role.

Circular swimming elicited by injection of GABA-ergic drugs is independent of eye movements, because the results of the injection experiment in medaka from which the eyes had been removed, were the same as the results for intact medaka.

In the rat, unilateral, intrathalamic injection of muscimol into the ventromedial nucleus elicited ipsilateral circling behavior only with administration of apomorphine (dopamine agonist) (Di Chiara et al., 1979). In the medaka, unilateral injection of muscimol in the lateral posterior thalamus elicited contralateral circular swimming without administration of apomorphine. Clarification of the mechanisms responsible for these differences remains for future study.

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メダカ視床後部へのGABA-アゴニストおよびアンタゴニストの微量注入によって得られる円型遊泳

竹内邦輔

メダカの遊泳型と脳の関係を知るためにこの実験は行われた．左視床後外側部にGABAのアンタゴニストであるビククリンを注入すると，メダカは左旋回性の円型遊泳をするようになる．また同じ場所にGABAのアゴニストであるムシモルを注入すると右旋回性の円型遊泳をするようになる．その他のGABA関係のアンタゴニスト，アゴニストも同様の影響を持っている．しかしGABA以外の神経伝達物質のアンタゴニストにはほとんど影響されない．このことから，メダカでは視床後外側部にGABA抑制性の神経による左右旋回を支配しているセンターの一つがあるように思われる．またそのリセプターについてはGABA-A型リセプターの方がGABA-B型リセプターより強く関係していると思われる．

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