Mem. Fac. Lit. & Sci., Shimane Univ., Nat. Sci., 4, pp. 153–163,
11 text-figs., 2 tables, March 15, 1971

Effects of Monoamine Oxidase Inhibitors and Pyrogallol on the Pigment Aggregating Response of the Fish Melanophores

Tetsuro IGA

Department of Biology, Shimane University, Matsue, Japan
(Received October 13, 1970)

It is generally accepted that in many teleosts the melanophores are primarily controlled by the autonomic nervous system, and that the nerve functioning in pigment aggregation is sympathetic. Many physiological and pharmacological data have shown that the transmitter released from the nerve endings is adrenergic and that the receptor in the melanophore may be of the *alpha* nature (Fujii 1961, Scheline 1963, Scott 1965, Abbott 1968, Iga 1968a, Fujii and Novales 1969). No matter what nature of the neurotransmitter acting at the neuro-melanophoral junction may be, the neurotransmitter suffers finally enzymatic destruction and must be removed from the sites of action, after the released, active neurohumore reacted with the receptor and played some roles as a trigger of excitation.

Participation of the following two kinds of enzymes is thought to be important on the metabolism of catecholamines : one is monoamine oxidase (MAO) acting on deamination of the amines, and the other catechol-O-methyltransferase (COMT) catalyzing O-methylation of the catecholamines. It has been generally believed that O-methylation by COMT is the first and important step in mammals (Kopin and Axelrod 1963, Axelrod 1965, Nagatsu 1965, Yoshida 1966) : Inhibition of MAO activity generally failed to augment the effect of the catecholamine, but COMT inhibitors induced the augmentation of the effect of endogenous and exogenous adrenaline. However, Crout *et al.* (1961) have been reported that the relative significance of these two metabolic pathways probably varies considerably from organ to organ and from species to species. Furthermore, there are some reports that the action of tyramine is potentiated by the MAO inhibitors (Giersemer *et al.* 1953, Goldberg and Sjoerdsma 1959, Vanov 1962). The mechanism of tyramine potentiation after the treatment of MAO inhibitors is not always clear, because tyramine acts indirectly through release of endogenous catecholamines on the target cells.

On the other hand, on physiology of the fish melanophores, there is little information from the view point of the inactivation process of the transmitter substance except pharmacological studies of Scott (1965, 1968), who observed the potentiating effect of norepinephrine and epinephrine injected subcutaneously with pretreatment of pyrogallol, an inhibitor of catechol-O-methyltransferase, in the sand flounder *Scopthalmus aquosus*.

The present experiments were designed to examine whether the MAO inhibitors can modify the pigment aggregating effect of the endogenously released neurohumore and also exogenous adrenaline. Furthermore, the similar experiments were carried out with

pyrogallol and the results were compared with those with MAO inhibitors. Thus, an attempt was made to study the problem of inactivation of the pigment aggregating neurotransmitter in the fish melanophore and to ascertain the nature of the transmitter by an indirect pharmacological approach.

Materials and Methods

The experiments were carried out exclusively on the scale melanophores of *Oryzias latipes* (the wild type) of body lenght 25-35*mm*. A scale isolated from the fish was fixed in position under a cover glass, epidermal side down, on a glass trough for microscopic observation, which was filled with physiological saline.

The physiological saline solution had the following composition : NaCl 128 *mM*, KCl 2.6 *mM*, $CaCl_2$ 1.8 *mM*, pH 7.2 by $NaHCO_3$. Isotonic (*M*/7.5) solution of KCl was also buffered with $NaHCO_3$ (pH 7.2). The "K solution" which contained *M*/7.5 KCl and *M*/7.5 NaCl in various proportions was used, in order to obtain an adequate value of pigment aggregation of the melanophore. For this purpose, 1.5 K solution was mainly used in the experiments. The 1.5 K solution refers to the external solution in which *M*/7.5 KCl occupies 1.5 parts of the total 10 in volume, the remaining 8.5 parts being *M*/7.5 NaCl. Adrenaline (Adrenalin Hydrochloride, 1 : 1000, Sankyo Co.) was freshly prepared in the physiological saline. The following monoamine oxidase inhibitors were used : phenelzine(β-phenylethyl hydrazine sulfate), safrazine(β-piperonyl isopropyl hydrazine hydrochloride) and nialamide (N-isonicotinoyl-N'-〔β-(N-benzyl-carboxamido) ethyl〕 hydrazine). Pyrogallol was dissolved in the physiological saline immediately before use.

Drugs were applied on the melanophores as external solution by changing the physiological solution to various experimental fluids. The magnitude of the response was given as the degree of pigment aggregation of a single melanophore : the magnitude of pigment aggregation is expressed by a shortened length of a branch in percentage of the length in full expansion, the amount in complete shortening being taken as 100.

The scale was immersed in the physiological solution for 15 minutes after isolation. The K solution or adrenaline solution was applied at first for 10 minutes, in order to determine the exact extent of response to the test solution. After this treatment the external solution was changed again to the physiological solution and the preparation was kept in it for 15 minutes. Then, the K solution or adrenaline solution containing one of the enzyme inhibitors in various concentrations was applied for 10 minutes. The effect of the test solution containing an enzyme inhibitor was compared with that of the K solution or adrenaline solution which did not contain the enzyme inhibitor. As criterion for potentiation or reduction of the aggregating effect of potassium ions or adrenaline, the difference over 10% in the aggregating value to the test solution and to that containing the enzyme inhibitor was taken as a level of significance.

As the responsiveness of the melanophores largely depends on the temperature, a certain series of experiments was taken care to be carried out at room temperature within certain limted ranges. The temperature during the experiments was listed in Tables 1 and 2.

Results

1. *Actions of monoamine oxidase inhibitors on the melanophores*

Actions of three monoamine oxidase inhibitors on the melanophores were investigated. The test solution was continuosly applied on the melanophores for 30 minutes or more. Phenelzine showed a marked pigment aggregating action to the innervated melanophores. The threshold concentration for pigment aggregation was about 5×10^{-7} *M*. At a high concentration (10^{-3} *M*), this drug induced rapid and transient pigment aggregation of the melanophores and then pigment dispersal took place. Recovery to

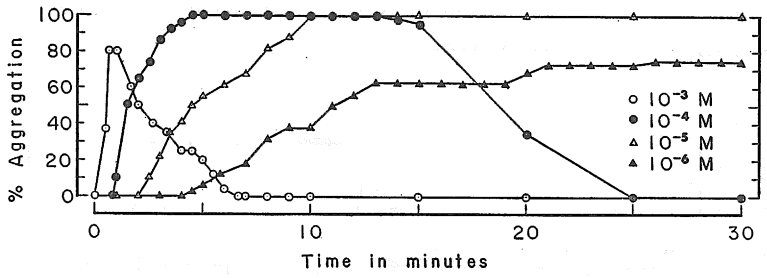


Fig. 1. Typical responses of innervated melanophores to phenelzine (Nardil) in various concentrations. Room temp.: 24-28°C.

the original dispersed state required usually several minutes. Figure 1 shows the time graphs of the responses of the innervated melanophores to the treatment with phenelzine in various concentrations. Once the redispersion of the pigment in the melanophores was attained with the treatment of phenelzine, the melanophores became inactive to KCl. The pigment aggregating effect of phenelzine was not observed on the denervated melanophores. Safrazine, at a high concentration (10^{-3} *M*), induced in some cases pigment aggregation of the melanophores. In these cases, pigment redispersion took place after the slight aggregating response. Nialamide ($10^{-3}-10^{-6}$ *M*) was inactive on pigment aggregation.

The experiments were carried out at room temperature within a range of 24−28°C.

2. *Effects of monoamine oxidase inhibitors and pyrogallol on the pigment aggregating response to potassium ions*

KCl induces pigment aggregation of the melanophores by acting selectively on the concentrating nerve endings and making release of a transmitter substance (Fujii 1959, Iwata *et al.* 1959).

The experiments were designed to investigate whether the action of the endogenously released pigment aggregating neurohumore is modified with monoamine oxidase inhibitors and also with pyrogallol. The 1.5 K (or 2 K) solution was used as an appropriate submaximal strength of stimulation. First, a preparation was immersed in the K solution. The treatment was continued for 10 minutes. Then, the perfusing solution was changed to the physiological solution and the preparation was kept in it for 15 minutes. The melanophores fully recovered their original dispersed states within several

minutes. Next, the application of the K solution containing an enzyme inhibitor, such as phenelzine, lasted for 10 minutes as had been the case with the K solution. In calculating the results, the effect of the first application of the K solution was considered as the control. The magnitude of the response to the first application of K solution was compared with that to the application of K solution containing an enzyme inhibitor. The values of pigment aggregation by the repeated applications of the K solution did not alter significantly (Figs. 3 and 6).

Phenelzine, being used almost below the threshold concentration for pigment aggregation, in 10^{-6} *M* and 5×10^{-7} *M* augmented the action of K ions. However, the augmentation effect was not observed in 10^{-7} *M*. Figure 2 showed an experiment where phenelzine augmented the action of K ions. Safrazine and nialamide did not modify the aggregating action of K ions.

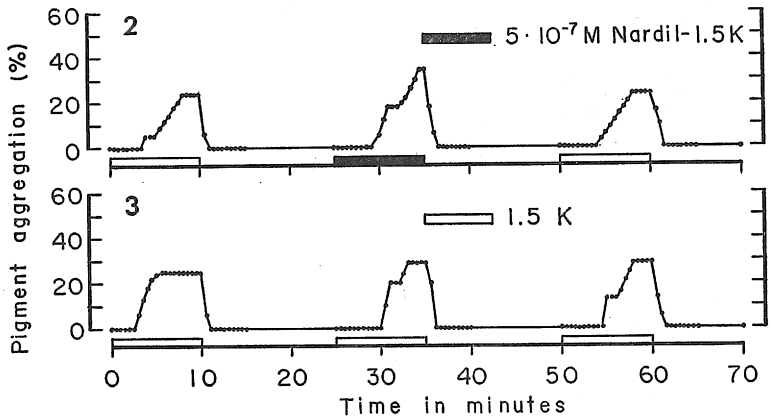
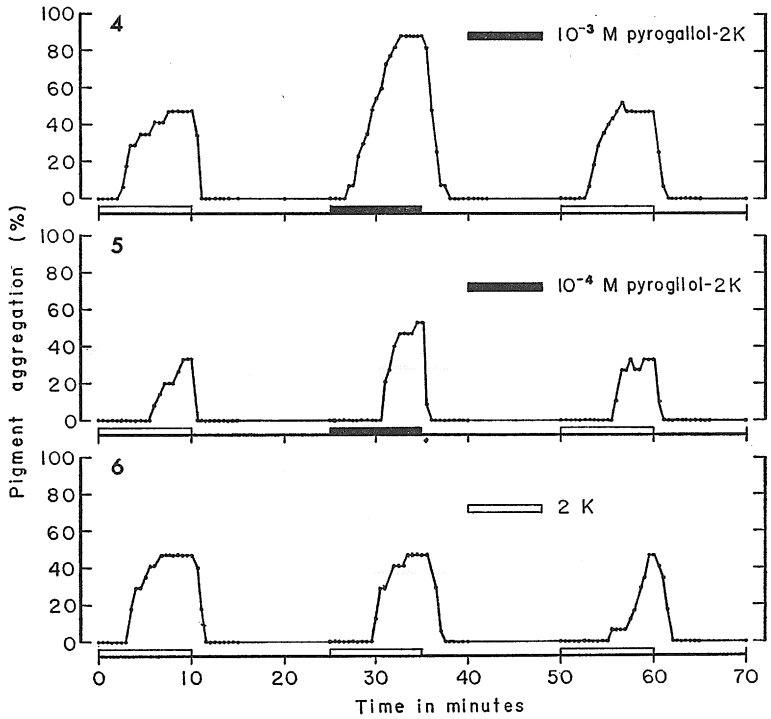


Fig. 2. Effect of phenelzine on the pigment aggregating response to KCl. Room temp., 18°C. Fig. 3. Repetitive application of 1.5 K solution. Room temp.: 21°C.

On the other hand, the action of K ions was significantly augmented by pyrogallol, when given simultaneously with K ions, in most experiments. Pyrogallol, in high concentrations, caused in some cases slight pigment aggregating effect when given alone, but the effect was small or not apparent at the concentration levels used to augment K activity. Figures 4 and 5 represented the typical examples showing the effect of pyrogallol. Table 1 summarized in general the results obtained on the effects of MAO inhibitors and pyrogallol. The last column of the Table shows the similarity of aggregating value of the melanophores to 1.5K (or 2 K) solution on the repeated applications as control experiments. In this case, the value of aggregation on the first application of the K solution was compared with that on the second application (see Figs. 3 and 6).



Figs. 4 and 5. Augmentation of the pigment aggregating response to KCl by pyrogallol. Fig. 6. Repetitive application of 2 K solution, shown as a control experiment. Room temp.: 17-18°C.

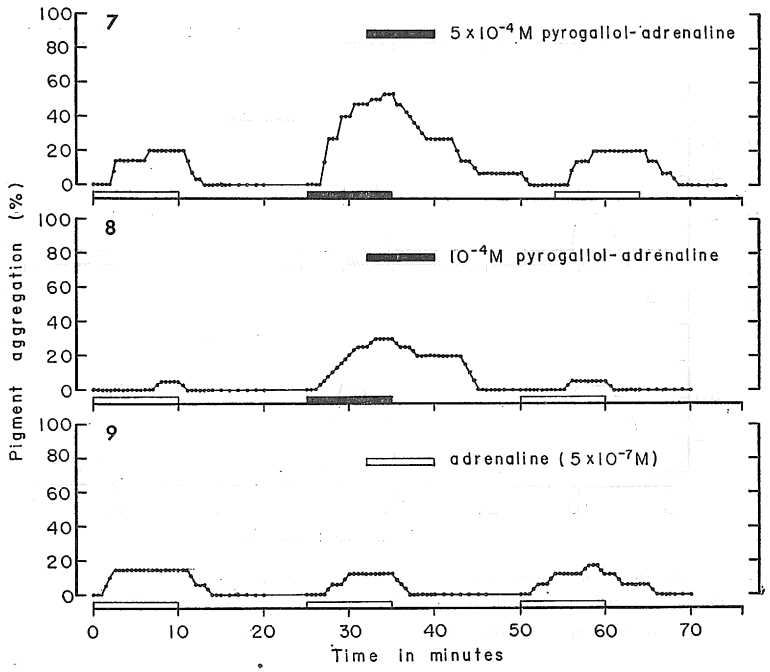
Table 1
Effects of MAO inhibitors and pyrogallol on the melanophore aggregating response to KCl

Inhibitor	Concentration of inhibitor (M)	Effect +	0	−	No. Exp.	Temp. °C.
Phenelzine	10^{-6}	5	8	0	13	17−22
	5×10^{-7}	7	16	1	24	
	10^{-7}	1	12	0	13	
Safrazine	10^{-4}	0	9	3	12	20−23
	10^{-5}	2	8	1	11	
	10^{-6}	1	6	3	10	
Nialamide	10^{-4}	4	14	3	21	18−20
Pyrogallol	10^{-3}	11	2	0	13	16−19
	5×10^{-4}	11	2	0	13	
	10^{-4}	5	5	0	10	
(Control)	1.5 K	1	23	3	27	17−22
	2.0 K	2	11	3	16	16−19

+, augmented effect; −, reduced effect; 0, effect is not modified.

3. *Effects of monoamine oxidase inhibitors and pyrogallol on the pigment aggregating response to adrenaline*

Effects of enzyme inhibitors on the pigment aggregating response to exogenous adrenaline were examined by the same procedure as described in the previous section.



Figs. 7 and 8. Augmentation of the pigment aggregating response to adrenaline by pyrogallol. Fig. 9. Repetitive application of adrenaline. Room temp.: 18, 16 and 15°C, respectively.

Table 2

Effects of MAO inhibitors and pyrogallol on the melanophore aggregating response to adrenaline

Inhibitor	Concentration of inhibitor (M)	Effect +	0	−	No. exp.	Temp. °C.
Phenelzine	10^{-6}	11	0	0	11	19−22
	5×10^{-7}	3	8	0	11	
	10^{-7}	1	10	0	11	
Safrazine	10^{-5}	2	10	2	12	20−22
Nialamide	10^{-4}	2	8	2	12	19−21
Pyrogallol	10^{-3}	11	0	0	11	15−18
	10^{-4}	10	1	0	11	
	5×10^{-5}	9	2	0	11	
(Control)	5×10^{-7} Adrenaline	1	26	2	29	15−20

+, augmented effect; −, reduced effect; 0, effect is not modified.

5×10^{-7} M adrenaline was mainly used. The adrenaline solution induced slight aggregation of the pigment in the innervated melanophores in most experiments. Table 2 shows in general the results obtained on the effects of the MAO inhibitors and pyrogallol on the response to exogenous adrenaline. Phenelzine in 10^{-6} M augmented the action of adrenaline. The potentiating effect was small in the case of 5×10^{-7} M phenelzine. Safrazine and nialamide could not produce the potentiation of the action of adrenaline. Pyrogallol, when given simultaneously with adrenaline, markedly and significantly augmented the action of adrenaline in almost all experiments. This potentiation was apparent in both the aggregating value and in duration of the effect. The typical examples of pyrogallol were represented in Figures 7 and 8, and a typical example on the repetitive applications of adrenaline as control experiments in Figure 9.

Discussion

In their experiment designed to examine the effects of several monoamine oxidase inhibitors to the cardiovascular actions of the various amines in the dog, Goldberg and Sjoerdsma (1959) reported that JB-516 (1-phenyl-2-hydrazinopropane HCl) and JB-835 (4-phenyl-2-butyl-hydrazine HCl), two of six MAO inhibitors used, possess the marked sympathomimetic actions. Similar sympathomimetic effect was obtained on the fish melanophores by Scott (1965, 1968). He found out that in addition to many "sympathomimetic amines", phenelzine and pheniprazine, which are in the MAO inhibitor category, induced pigment aggregation of the melanophores in the sand flounder when these drugs were injected subcutaneously. Phenelzine acted as a drastic pigment aggregator also in the present experiments using the isolated scale melanophores. Safrazine and nialamide were inactive. The chemical structure of phenelzine and also of pheniprazine resembles that of β-phenylethylamine, which causes pigment aggregation of the melanophores, acting on the endings of the pigment concentrating nerve (Iga 1968b). These experimental data suggest that pigment aggregation by phenelzine is probably unrelated to its MAO inhibiting property, and that the aggregating action may be ascribable to the common chemical homology as sympathomimetic amines, such as β-phenylethylamine and ephedrine.

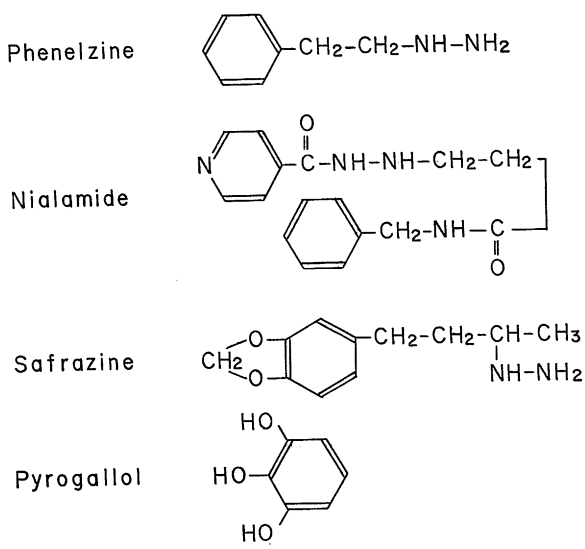


Fig. 10. Chemical structures of the drugs used.

An another experiment was tried to decide the action point of phenelzine as a pigment aggregator. The action of the drug

disappeared in the denervated melanophores. The fact shows that phenelzine does not act directly on the melanophore itself, but acts indirectly through intervention of the aggregating nerve. Furthermore, an experiment of local application of the drug indicated that phenelzine may act on the endings of the aggregating nerve ; the aggregating response of the melanophores was restricted only within an area of application of the drug (Fig. 11). The procedure of drug application was the same with the one previously used (Iga 1968b).

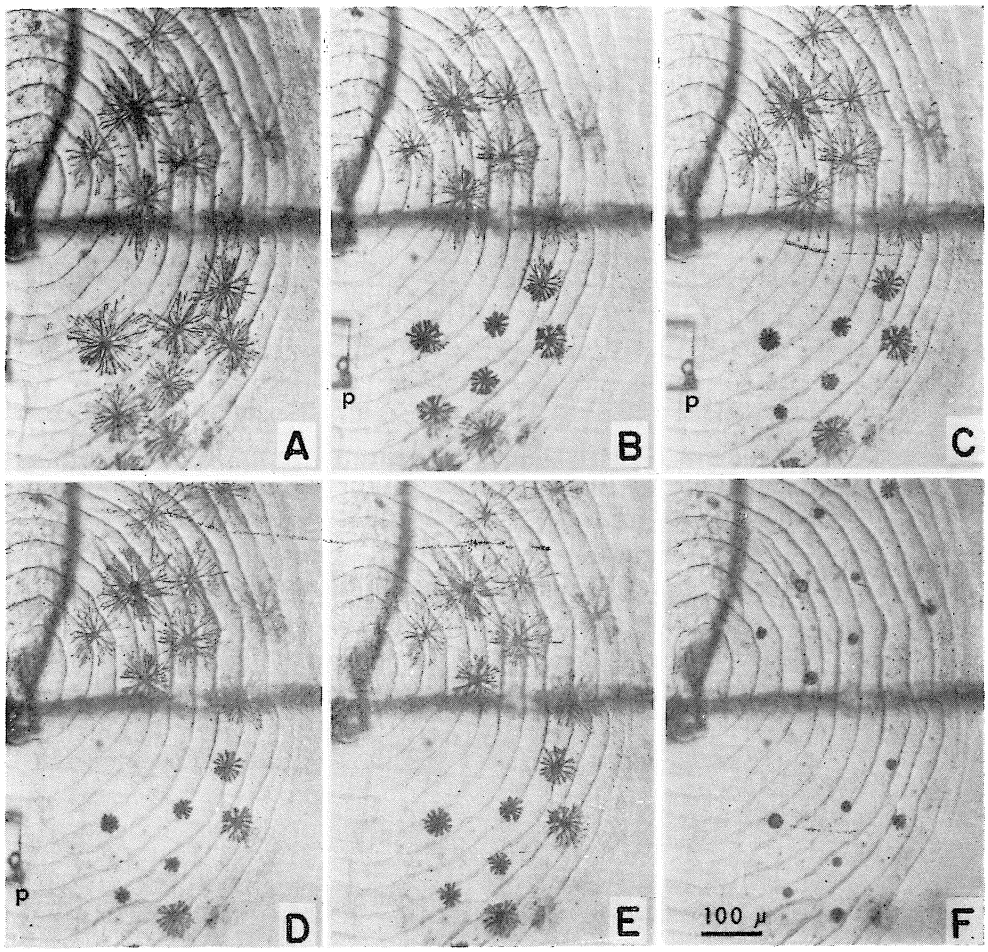


Fig. 11. Local application of phenelzine on the scale melanophores of *Oryzias latipes*. A : dispersion state in physiological solution. B, C and D : 1, 2 and 5 minutes after outflow of phenelzine (10^{-4} *M*), only the melanophores in the treated area respond in clear contrast to the remaining ones which remain in a condition of full dispersion. E : 5 minutes after the stop of outflow of the test solution. F : KCl application by exchange of the external fluid. p, micropipette for local application of the test solution. Room temp., 23°C.

It has been generally accepted that the MAO inhibition *in vivo* does not modify the effects of the injected adrenaline and noradrenaline (Griesmer *et al.* 1953, Goldberg and Sjoerdsma 1959, Vanov 1962). The enhancement of the effects of noradrenaline and adrenaline, observed occasionally, is not probably directly related to enzyme inhibition (Horwitz *et al.* 1960). In the present experiments, MAO inhibitors did not have constant and decided effects on the melanophore response to the endogenously released neurotransmitter and also to exogenous adrenaline : Phenelzine, in some cases, induced augmenting effects, but not safrazine and nialamide. Phenelzine itself had enough pigment aggregating activity to make interpretation of the results difficult. Thus, the augmentation may be unrelated to the inhibition of enzymatic activity, but rather be the results of the summation of the responses to the stimulant solution and phenelzine.

Pyrogallol has been observed to potentiate the action of endogenous and exogenous epinephrine in the rat (Wylie 1960). The present finding is interesting and significant on physiology of fish melanophore ; pyrogallol augmented the effect of endogenously released neurotransmitter and of exogenous adrenaline. Similar augmented effect was observed when pyrogallol was previously injected subcutaneously in the flatfish (Scott 1965, 1968). Scheline (1962) detected methoxy compounds in the water of the tank where the cod, *Gadus callarias*, is reared and pointed out the presence of the enzyme COMT in the fish.

The present experimental data by pyrogallol are consistent with current concepts that efficient routes of metabolism of catecholamine *in vivo* may be O-methylation by COMT. The data also show an important participation of the enzyme COMT on inactivation of the transmitter concerning pigment aggregation of the melanophores. Furthermore, the presence of COMT would strengthen the probability of a catecholamine functioning as the normal transmitter at the neuro melanophoral junction.

From these conclusions, it would be especially significant that methylated derivatives of adrenaline and noradrenaline, metanephrine (3-O-methyladrenaline) and normetanephrine (3-O-methylnoradrenaline), were several hundred to thousand time less active than the genuine amines on melanophore pigment aggregating activity (Iga 1968c).

Summary

The effects of three monoamine oxidase (MAO) inhibitors (phenelzine, safrazine and nialamide) and pyrogallol on the melanophore pigment aggregating action of potassium ions and adrenaline were investigated by using the isolated scale preparation of *Oryzias latipes*.

Phenelzine itself possessed a marked aggregating action on the innervated melanophores, but was inactive on the denervated preparations. The experiment of local application of phenelzine to the innervated melanophores suggested that this drug acts on the terminals of the pigment aggregating nerve.

The pigment aggregating action of phenelzine was probably unrelated to the MAO inhibiting property of the drug, because safrazine and nialamide did not possess the aggregating effect.

Phenelzine, when given simultaneously with potassium ions or adrenaline, augmented

the action of potassium ions and adrenaline, while safrazine and nialamide did not alter significantly the effect of K ions and of adrenaline. The augmented effect by phenelzine was discussed in relation to the similarity of the chemical structure between phenelzine and some sympathomimetic amines.

Pyrogallol potentiated significantly the action of K ions and of adrenaline.

These results suggest an active participation of the enzyme catechol-O-methyltransferase (COMT) in the melanophore system in *Oryzias latipes*. The presence of the enzyme would strengthen that the transmitter functioning in pigment aggregation of the melanophores is adrenergic.

Acknowledgments

The author wishes to express his gratitude to Professor H. Nagahama of Hiroshima University for his invaluable advice. Many thanks are also due to Ono pharmaceutical Co. for supplies of penelzine (Nardil) and safrazine (Safra), and Taito Pfizer Co. for nialamide (Niamide)

Literature

Abbott, F. S. (1968) The effects of certain drugs and biogenic substances on the melanophores of *Fundulus heteroclitus*. Cand. J. Zool., 46 : 1149-1161.

Axelrod, J. (1965) The metabolism, storage, and release of catecholamines. Rec. Pro. Hormone Res., 21 : 597-622.

Crout, J. R., Creveling, C. R. and Udenfriend, S. (1961) Norepinephrine metabolism in rat brain and heart. J. Pharmacol. exp. Therap., 132 : 269-277.

Fujii, R. (1959) Mechanism of ionic action in the melanophore system of fish I. Melanophore-concentrating action of potassium and some other ions. Annot. Zool. Japon., 32 : 47-58.

_____, (1961) Demonstration of the adrenergic nature of transmitter at the junction between melanophore-concentrating nerve and melanophore in bony fish. J. Fac. Sci. Univ. Tokyo, IV, 9 : 171-196.

Fujii, R. and Novales, R. R. (1969) Cellular aspects of the control of physiological color changes in fishes. Am. Zoologist, 9 : 453-463.

Goldberg, L. I. and Sjoerdsma, A. (1959) Effects of several monoamine oxidase inhibitors on the cardiovascular actions of naturally occurring amines in the dog. J. Pharmacol. exp. Therap., 127 : 212-218.

Griesemer, E. C., Barsky, J., Dragstedt, C. A., Wells, J. A. and Zeller, E. A. (1953) Potentiating effect of iproniazid on the pharmacological action of sympathomimetic amines. Proc. Soc. Exp. Biol. & Med., 84 : 699-701.

Horwitz, D., Goldberg, L. and Sjoerdsma, A. (1960) Increased blood responses to dopamine and norepinephrine produced by monoamine oxidase inhibitions in man. J. Lab. Clin. Med., 56 : 747-753.

Iga, T. (1968a) Action of catecholamines on the melanophores in the teleost, *Oryzias latipes*. Zool. Mag., 77 : 19-26.

_____, (1968b) Action of *beta*-phenylethylamine on the melanophores in a teleost, *Oryzias latipes*. Mem. Fac. Lit. & Sci., Shimane Univ., Nat. Sci., 1 : 63-67.

_____, (1968c) Action of normetanephrine and its related compounds on the melanophores in a teleost, *Oryzias latipes*. Zool. Mag., 77 : 141-145.

Iwata, K. S., Watanabe, M. and Nagao, K. (1959) The mode of action of pigment concentrating agents on the melanophores in an isolated fish scale. Biol. J. Okayama Univ., 5 : 195-206.

Kopin, I. J. and Axelrod, J. (1963) The role of monoamine oxidase in the release and metabolism of norepinephrine. Ann. N. Y. Acad. Sci., 107 : 848-855.
Nagatsu, T. (1965) Biosynthsis and metabolism of catecholamines. *Seikagaku*, 37 : 697-715.
Scheline, R. R. (1962) O-methylation in fish. Nature, 195 : 904-905.
_____, (1963) Adrenergic mechanism in cuckoo wrasse, *Labrus ossifagus*, L. Comp. Biochem. Physiol., 9 : 215-227.
Scott, G. T. (1965) Physiology and pharmacology of color change in the sand flounder *Scopthalmus aquosus*. Limno. Oceano., 10 : 230-246.
_____, (1968) The action of phenothiazines on the pigment cells of the flatfish *Scopthalmus aquosus*. Agressologie, 9 : 3-11.
Vanov, S (1962) Effect of monoamine oxidase inhibitors and pyrogallol on the pressor response to adrenaline, noradrenaline, normetanephrine and tyramine. Arch. int. Pharmacodyn., 138 : 51-61.
Wylie, D. W., Archer, S. and Arnold, A. (1960) Augmentation of pharmacological properties of catecholamines by O-methyltransferase inhibitors. J. Pharmacol. exp. Therap., 130 : 239-244.
Yoshida, H. (1966) Inactivation processes of catecholamine. *Seikagaku*, 38 : 402-413.