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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 12 (1): 1-10, 2009
ISSN 1028-8880

Postsynaptic Alpha 2-Adrenoceptors Mediate Melanosome Aggregation in Melanophores of the White-Spotted Rabbitfish (*Siganus canaliculatus*)

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Abstract: The present investigation was undertaken to study the nature of neuro-melanophore junction in the white-spotted rabbit fish *Siganus canaliculatus*. *In vitro* experiments using split fin preparation indicated that melanophores of *S. canaliculatus* are highly responsive to potassium ions and adrenergic agonists. Potassium ions and the adrenergic agonists induced prompt melanosome aggregation that could be competitively blocked by yohimbine (alpha-2 specific adrenergic antagonist) and phentolamine (non-specific alpha adrenergic antagonist). The melanophore responses to repeated potassium stimulation (up to 20 stimuli) did not show any sign of fatigue. However, statistically significant enhancement was observed in responses to potassium that followed the first five stimulations. Adrenergic agonists acted in a time and concentration-dependent manner and their relative potency had the following rank order: clonidine (alpha-2 specific agonist) > norepinephrine (non-specific adrenergic agonist) > phenylephrine (alpha-1 specific agonist) > methoxamine (alpha-1-specific agonist). Yohimbine exerted a more potent inhibiting effect on norepinephrine induced melanosome aggregation compared to phentolamine. Prazosine (alpha-1 specific antagonist) had no effect on such aggregation. Chemically denervated melanophores displayed hypersensitivity to alpha-adrenergic agonists but were refractive to potassium ion stimulation. The refractivity of denervated melanophores to potassium indicates the effect of potassium ion is not direct on melanophores but it is rather through depolarization effect of potassium on the neuro-melanophore peripheral sympathetic fibers and hence release of norepinephrine. In denervated melanophores, similar to intact melanophores, only phentolamine and yohimbine but not prazosine, significantly inhibited melanosome aggregation effect of norepinephrine, indicating that norepinephrine effect is through postsynaptic alpha-2 adrenoceptors. The present data demonstrate that the nature of melanophore innervation in this teleost is adrenergic and neuro-melanophore signals mediating melanosome aggregation are transmitted through alpha-2 postsynaptic adrenoceptors.

Key words: Pigment cell, pigment granule, pigment aggregation, adrenergic receptor, melanophore, hypersensitivity, agonists, antagonists, denervation

INTRODUCTION

Physiological and pharmacological studies on the responses of teleost melanophores have disclosed that the nature of peripheral neurotransmission mediating the aggregation of the melanosome into the perikaryon of the cell is adrenergic (Fujii, 2000). It is now well accepted that the transduction of neural signals for melanosome aggregation in teleost is mediated through postsynaptic alpha adrenoceptors and the involved neurotransmitter is naturally considered to be norepinephrine (Fujii and Novales, 1972; Fujii, 1993). It has also been clearly demonstrated that the presence of calcium ions is essential for the release of the mediating neurotransmitter (Fujii and Oshima, 1986; Katayama *et al*., 1990; Visconti and Castrucci, 1990).

On the other hand, there is considerable variation among and within fish species in melanophore responses to different stimuli. This variation in responses has been attributed mainly to the differences in the density of melanophore innervation and the nature and density of receptors mediating melanosome motility (Kasukawa *et al*., 1985; Miyata and Yamada, 1987). Furthermore, studies involving different species of teleost, have indicated that postsynaptic adrenoceptors mediating melanosome aggregation are more probably of alpha-2 subtype (Andersson *et al*., 1984; Karlsson *et al*., 1987). A possible involvement of alpha-1 in mediation of melanosome aggregation has been also speculated. Burton and Vokey (2000) investigating the responses of *Pleuronectes americanus* patterns to K^+-rich saline and adrenergic agonists and antagonists, have reported a significant role of alpha-1 adrenoceptors in the mediation of melanosome aggregation particularly in melanophores of white spots of this fish.

The white-spotted rabbitfish *Siganus canaliculatus* that belongs to the family Siganidea exhibits distinct color patterns. With a touch of olive green on nape and upper surface of head, the body hue from the above is silvery gray and from the below is silver (Musaiger and D'Souza, 2008). During preliminary observations, it was noticed that the fish changes its patterns very promptly in response to any disturbances in the surroundings or changes in the back ground. Thus indicating the presence of an efficient neuro-chromatophore regulating mechanisms. Since, no literature could be traced on the chromatic responses of *S. canaliculatus*, the present study was undertaken to reveal the nature of neuro-melanophore junction of this teleost and to study the possibility of its implementation as a miniature model in physiological and pharmacological studies related to the mechanisms involved in the regulation of neuro-effector junction at the tissue level as well as cellular and sub-cellular levels.

MATERIALS AND METHODS

The present study was conducted during academic year 2006/2007 at the Marine Resources Research Center, Um AL Quwain, Ministry of Environment and Agriculture, UAE. Irrespective of their sex, adult *Siganus canaliculatus* (Fig. 1) were kept at least for three days of acclimatization in aquariums supplied with running sea water before experimental use. Average weight and length of the fish was 70.9 g and 21.2 cm, respectively (n = 12). The specimens for studying the responses of melanophores, were prepared by split caudal fin technique as was described by Fujii (1959). The two halves of the split fin while immersed in a balanced physiological saline for teleosts of the following composition (mM): NaCl, 125.3; KCl, 2.7; $CaCl_2$, 1.8; $MgCl_2$, 1.8; (R)-(+)-Glucose, 5.6; Tris-HCl buffer, 5.0 (pH 7.2), were cut into pieces of 3-5 mm in size containing at least, almost, equal parts of two parallel fin rays with their intervening piece of skin. The specimens were mounted (epidermal side down) on a cover-slip under two pairs of parallel glass rods adhered from one side to the glass surface. A perfusion chamber (made of microscopic glass slide with suitable glass boundaries providing sufficient depth for liquid perfusion) was based on the stage of an inverted microscope with attached camera (Zeiss IM35). The specimens unless being exposed to one of the test solutions, they were continuously irrigated by the physiological saline.

In time-response experiments, response of a single melanophore was estimated using MI (Melanophore Index) as was introduced by Hogben and Slome (1931)

Fig. 1: White-spotted Rabbitfish (*Siganus canaliculatus*) exhibiting different pigment patterns

was used. Designating MI 1 to state of full melanosome aggregation and MI 5 is designated to the state of fully dispersed melanosomes. In cumulative concentration-response experiments, the percentage change in the apparent length of a particular dendrite process of a cell under observation was estimated using an ocular micrometer.

To study the effect of potassium ions [K^+], K^+-rich saline (K^+ = 50 mM) was used. The K^+-rich was prepared by elevating the K^+concentration in the original saline and balanced once again by an equimolar decrease in the concentration of Na^+, thus, the final osmolarity remained unchanged.

In experiments designed to study responses of denervated melanophores, the specimens were treated for 10 min with 10 μM reserpine dissolved in physiological saline containing 125 mM citric acid.

The relationship between concentration of an agonist and the magnitude of the evoked response was studied by exposing the preparation to solutions of various strength in cumulative and in increasing order.

The (log EC_{50}) value of each agonist was calculated from its concentration-response curve.

The following drugs supplied by Sigma (St. Louis, USA) were used: L-norepinephrine hydrochloride, L- epinephrine bitarterate, phenylephrine hydrochloride, clonidine hydrochloride, methoxamine hydrochloride, toazoline hydrochloride, yohimbine hydrochloride, prazosin hydrochloride and reserpine hydrochloride. Stock solutions of the drugs were prepared in physiological saline and were diluted with the same to the desired concentration when needed. All the experiments were conducted at room temperature that ranged from 20-24°C.

Statistical methods: The data reported in the present work (Mean±SE) were statistically analyzed using one-way analysis of variance (ANOVA), followed by Student Newman-Keuls post-hoc test. A p-value <0.05 was considered significant.

RESULTS

Effects of potassium ions: Under continuous perfusion of the physiological saline, *S. canaliculatus* melanophores assumed state of full melanosome dispersion. When the physiological saline was substituted with the K^+-rich saline (50 mM K^+), a prompt and complete melanosome aggregation was observed (MI changed from 5 to 1 in 30±5 sec, n = 6 different preparations). Substitution of K^+-rich saline with the physiological saline evoked complete redispersion of melanosomes (MI changed from 1 to 5, in 40±5 sec, n = 6). Therefore, complete cycle (aggregation in response to potassium and redispersion in physiological saline had time duration with a mean value of (70±10 sec) (Fig. 2, 3). The effect of K^+ ions was repeatable and up to twenty consecutive cycles of K^+ stimulation experimented in the present study did not show any sign of fatigue. However, it was observed that responses that follow the first five cycles of potassium stimulation, display statistically significant enhancement ($p<0.05$). The duration of cycle of the first five cycles of potassium stimulation had a time duration with a mean value of (77±5 sec n = 6) compared to the mean value (60±5 sec n = 6) of each of the next five cycles that followed (Fig. 4).

To investigate effects of alpha adrenergic antagonist on the melanosome aggregating effect of K^+-rich saline, the preparations were treated first with one of the three antagonists (10 μM) for 5 min. During 5 min treatment of the specimen with any of the three blockers, no change in the values of MI was observed (Fig. 5). Thus, the state of melanosome dispersion in the physiological saline was not affected by the addition of the antagonist alone. Then, in the presence of one of the antagonists, the preparation was perfused with K^+-rich saline. In presence of phentolamine and yohimbine, melanosome aggregation effect of K^+-rich saline was completely blocked. The blockage was reversible by

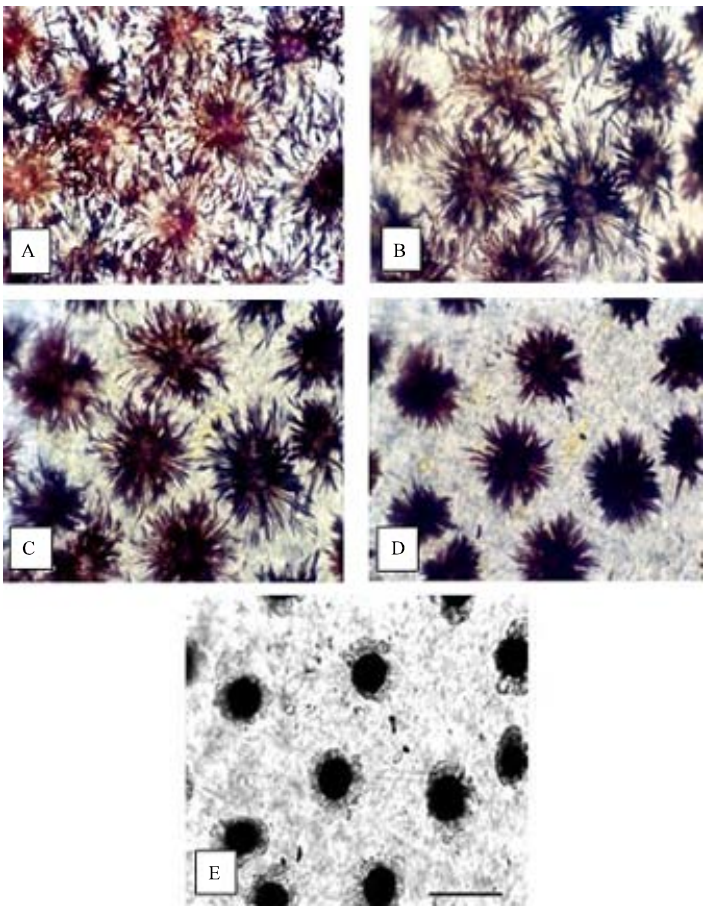

Fig. 2: A typical serial photomicrographs showing the effects of 50 mM K^+ ions on an a group of melanophores. A- Equilibrated in physiological saline. Melanophores are completely dispersed in the cell. B (15 sec), C (20 sec), D (25 s) and E (30 sec) after the application of 50 mM K^+ ions. Bar = 80 μm

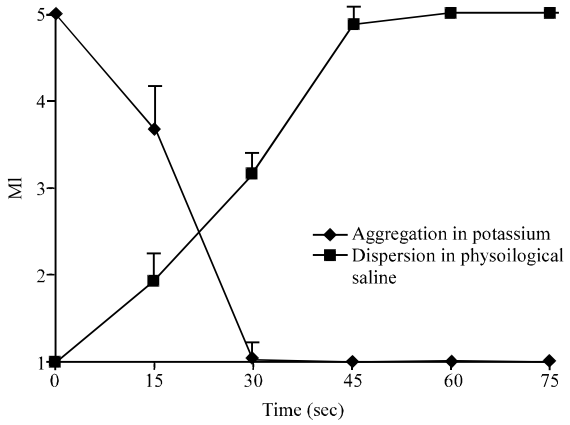

Fig. 3: Melanosome aggregation in response to K^+-rich saline and its redispersion in physiological saline after withdrawal of the former. Values = Means± SEM, n = 6

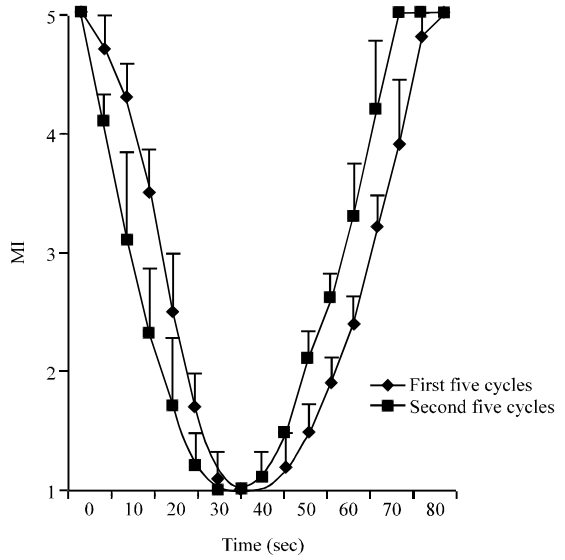

Fig. 4: Responses of melanophores to repeated consecutive K^+ stimulation. A significant enhancement is observed between the response of melanophores to the first five cycles of consecutive stimulation and the response of melanophores to the second five cycles of consecutive stimulation that followed. Values = Means±SEM, n = 6, $p<0.05$

re-perfusing the preparation with physiological saline. Prazosine at above concentration and even at 100 μM failed to inhibit melanosome aggregation effect of K^+-rich saline (Fig. 6).

Effects of adrenergic agonists: Administration of 500 nM norepinephrine evoked full aggregation of dispersed

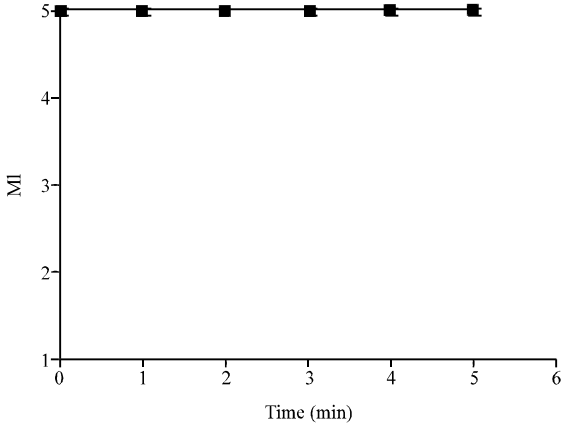

Fig. 5: Effects of alpha adrenergic antagonists (10 mM); yohimbine, phentolamine and prazosine. Administration of any of antagonist to the perfusion chamber for as long as 5 min did not induce any change in the status of fully dispersed melanosomes equliberated in physiological saline

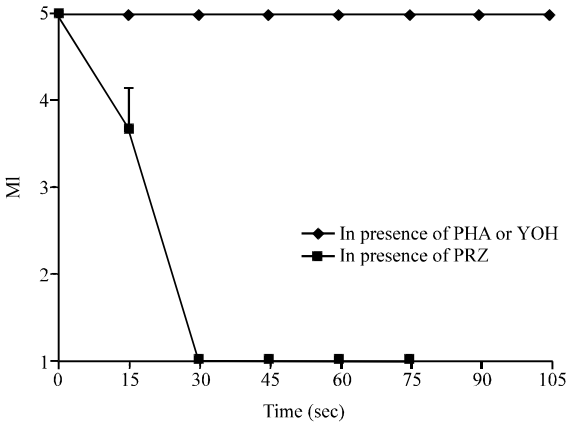

Fig. 6: Effects of prazosine (PRZ), phentolamine (PHA) and yohimbine (YOH) on melanosome aggregation effect of potassium. Values = Means±SEM, n = 6

melanosomes in preparations perfused with the physiological saline. The aggregation processes had a time duration with a mean value of 60 sec. The complete melanosome aggregation sustained during the 3 min administration of the agonist. Withdrawal of the agonist and the resumption of perfusion with the physiological saline resulted in melanosome dispersion that had a time duration with a mean value of 120 sec (Fig. 7, 8). Administration of norepinephrine in lower concentration than 500 nM did not evoke full melanosome aggregation. However, the rate of melanosome redispersion in the physiological saline was considerably slower if norepinephrine in concentration higher than 500 nM was used.

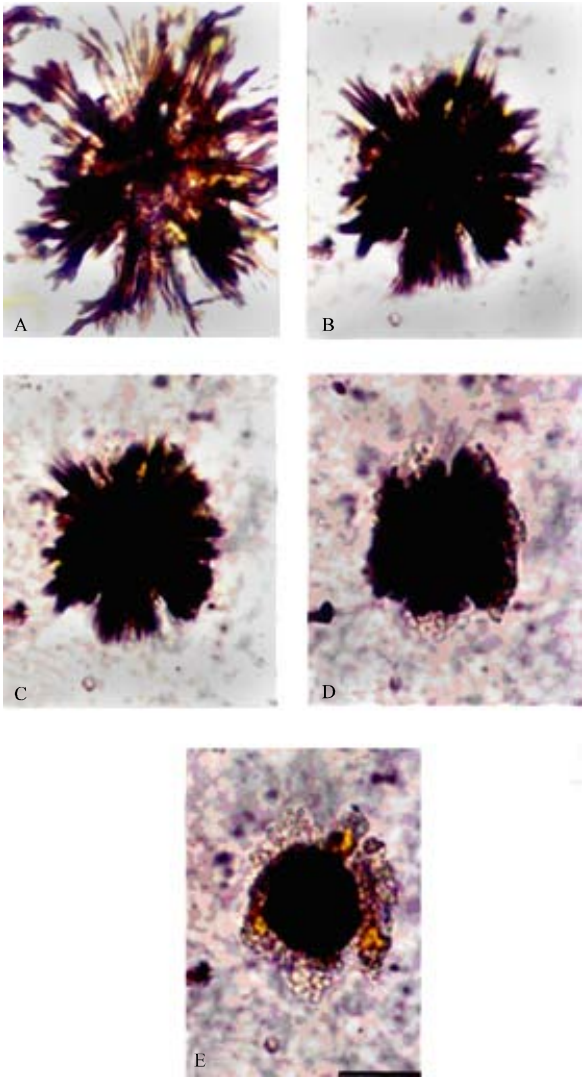

Fig. 7: A typical series of photomicrographs of single melanophore showing melanosome aggregation effect melanosome aggregation effect of norepinephrine. A-equilibrated in physiological saline. B (15 sec), C (30 sec), D (45 sec) and E (60 sec) after the administration of 500 nM norepinephrine. Bar = 150 μm

The magnitude and the rate of melanosome aggregation increased gradually as the concentration of norepinephrine administered were elevated, until the maximum response was attained. Thereafter, any additional elevation in concentration of the agonist did not result in any further increase in the rate or the magnitude of the response (Fig. 9).

Also other adrenergic agonists clonidine (alpha-2 specific), phenylephrine (alpha-1 specific), methoxamine

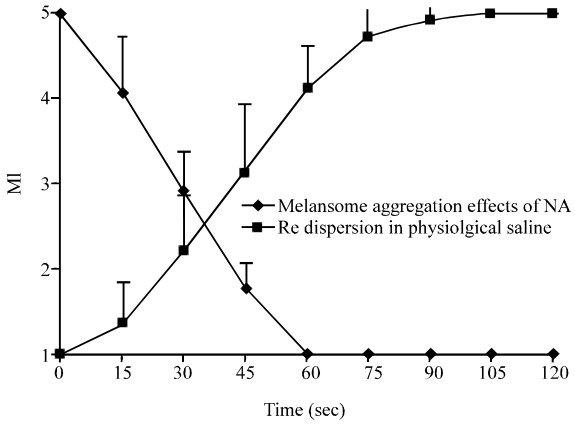

Fig. 8: Melanosome aggregation in response to norepinephrine (NE) (500 nM) and its redispersion in physiological saline after withdrawal of the former. Values = ±SEM, n = 6

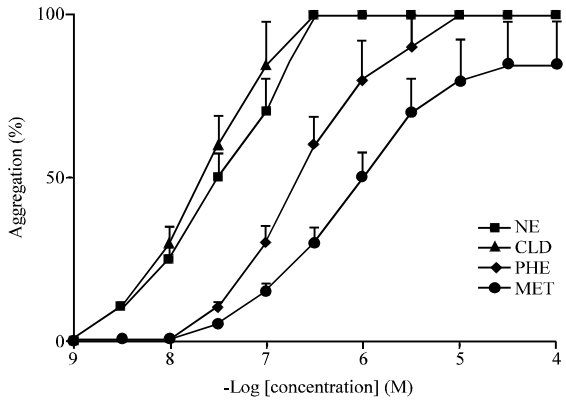

Fig. 9: Clonidine (CLD), norepinephrine (NE), phenylephrine (PHE) and methoxamine (MET) concentration response curves. Values = Means ±SEM, n = 6, p<0. 001 between agonist at EC_{50}

(alpha-1 specific) evoked melanosome aggregation within the melanophores. All the agonists induced melanosome aggregation in time and concentration-dependent manner. However, complete melanosome aggregation was not attainable by methoxamine. This is clearly demonstrated in the cumulative concentration-response (Fig. 9). Based on the EC_{50} values (values of negative logarithm of the molar concentration of the agonist that induced 50% of the maximal response), relative potencies of the agonists were estimated from the concentration-response curves (Fig. 9). The relative potency of agonists had the following rank of order: clonidine (EC_{50} = 30 nM) >norepinephrine (EC_{50} = 50 nM) >phenylephrine (EC_{50} = 300 nM) > methoxamine (EC_{50} = 950 nM).

Effects of adrenergic antagonists: In order to study the inhibitory effects of adrenergic antagonists on melanosome aggregation effect of norepinephrine, specimens before being subjected to the agonist were first pretreated for 5 min with one of the following alpha adrenergic antagonists: phentolamine (non-specific alpha antagonist), yohimbine (alpha-2 specific) and prazosin (alpha-1 specific). Then, while the concentration of antagonists (10 μM) was kept constant, various concentrations of norepinephrine were administered cumulatively and in increasing order. Yohimbine and phentolamine strongly inhibited melanosome aggregation effect of norepinephrine. The two antagonists shifted the concentration-response curve of norepinephrine to the right in parallel manner, indicating the competitive nature of inhibiting effects of the antagonists on the melanosome aggregation effect of norepinephrine. But, prazosine failed to exhibit any inhibition on melanosome aggregation effect of norepinephrie. Yohimbine exhibited greater inhibiting effect than phentolamine. EC_{50} in presence of yohimbine was shifted from 50 to 2500 nM while in presence of phentolamine EC_{50} was shifted from 50 to 750 nM (Fig. 10).

Effects of K^+-rich saline and adrenergic agonists and antagonists on denervated melanophores.

In these series of experiments, specimen subjected to chemical denervation, were first perfused (10 min) with the physiological saline. The affected melanophores were found to exhibit state of complete melanosome dispersion in the physiological saline. Substitution of the saline with K^+-rich saline did not bring about any change in the dispersed state of melanosome indicating the refractoriness of denervated melanophores to K^+-rich saline.

In contrast, denervated melanophores demonstrated significant enhancements in their responses to adrenergic agonists. The cumulative concentration-response curves for all the agonists were shifted to left. The enhanced responses of denervated melanophores to adrenergic agonist had the same rank of order as the responses of intact melanophores except for norepinephrine. Denervated melanophores were found to be more sensitive to norepinephrine than clonidine. Compared to intact melanophores, the sensitivity of denervated melanophores to norepinephrine increased by 76.9x while their sensitivity to clonidine increased only by 40x. Also denervated melanophores sensitivity to phenylephrine was enhanced by 12x and to methoxamine by 15.8x.

The relative potency of adrenergic agonists as estimated by EC_{50} had the following rank order: norepinephrine (EC_{50} = 0.55 nM)>clonidine (EC_{50} = 0.75 nM) > phenylephrine (EC_{50} = 25 nM)> methoxamine (EC_{50} = 60 nM) (Fig. 11).

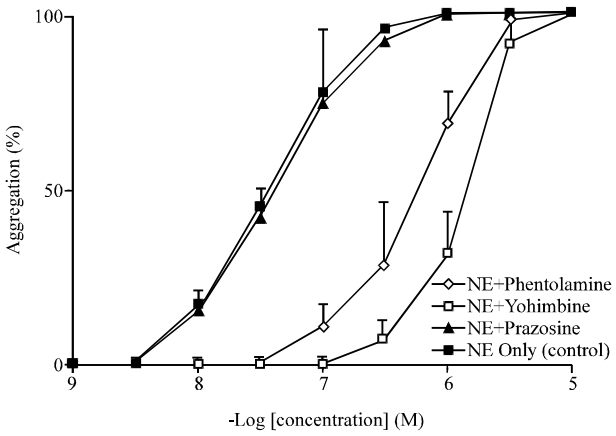

Fig. 10: Effects of alpha adrenergic antagonists (10 mM); yohimbine, phentolamine and prazosine on concentration response curve of norepinephrine (NE). Values = Means±SEM, n = 6. $p<0.005$ with yohimbine and phentolamine, $p>0.005$ with prazosine compared to control. $p<0.005$ between yohimbine and phentolamine at EC_{50}

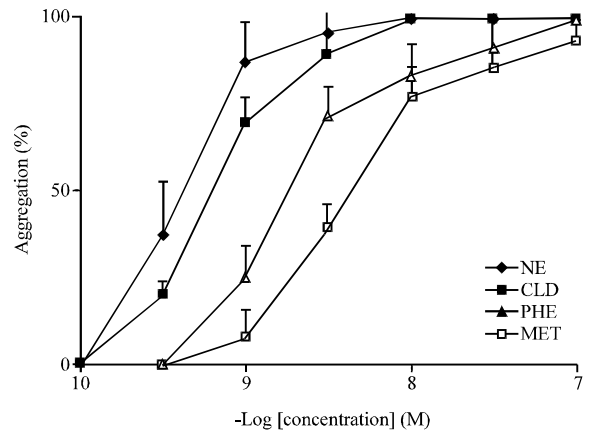

Fig. 11: Clonidine (CLD), norepinephrine (NE), phenylephrine (PHE) and methoxamine (MET) concentration response curves in denervated melanophores. Values = Means±SEM, n = 6, $p<0.005$ between EC_{50} (denervated) and EC_{50} (innervated) for all agonists

In order to study the effect adrenergic antagonist on the enhanced responses of denervated melanophores to norepinephrine, the specimen were first treated with 10 μM of one of the three antagonists (phentolamine, yohimbine, prazosine). Then, in presence of the antagonist, the preparation was perfused cumulatively and in increasing order with norepinephrine. In line with results obtained using intact melanophores, only

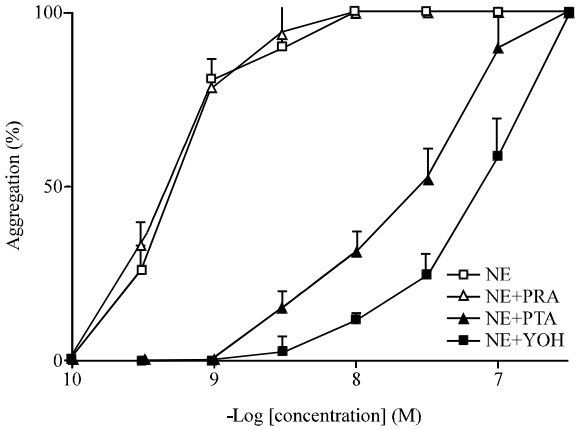

Fig. 12: Effects of alpha adrenergic antagonists (10 μM): NE (norepinephrine); PRZ (prazosine), PTA (phentolamine); YOH (yohimbine) on the concentration-response curve of melanosome aggregation effect of norepinephrine in denervated melanophores. Values = Means±SEM, n = 6

phentolamine and yohimbine, but not prazosine, displayed significant shift of concentration-response curve to the right. Also in denervated melanophores, yohimbine was found significantly to be a more potent blocker than phentolamine. The EC_{50} value in the presence of yohimbine changed from 0.55 to 90 nM and in the presence of phentolamine the EC_{50} value changed from 0.55 to 40 nM. Thus, the response sensitivity of denervated melanophores to norepinephrine in presence of yohimbine is reduced by 163.6x and in presence of phentolamine is reduced by 73x (Fig. 12).

DISCUSSION

The prompt and complete melanosome aggregations of *S. canaliculatus* melanophores in response to K^+ stimulation demonstrate that they are under control of nervous system. Moreover, the rate by which dispersed melanosomes in the physiological saline, aggregate in response to K^+ stimulation (30+5 sec) and their redispersal in the former after withdrawal of the later (40+5 sec), indicate that melanophores in this teleost are directly innervated and the peripheral pre-synaptic and post-synaptic mechanisms controlling the translocation of melanosomes are highly effective. Furthermore, the blocking effect of phentolamine and yohimbine, on the aggregating effect of K^+ and the refractoriness of denervated melanophores to K^+ stimulation, is well in line with the concept that the effect of K^+ is not direct, but it is rather through its depolarization effects on the peripheral sympathetic nerve elements and hence, the release of the neurotransmitter. The indirect effect of K^+ is now well accepted and their melanosome aggregating effect is considered to be mediated through the indigenous neurotransmitter (norepinephrine) acting on postsynaptic alpha adrenoceptors (Kumazawa and Fujii, 1984; Burton and Everard, 1991).

The potent melanosome aggregation induced by norepinephrine (60 sec) and other adrenergic agonists in melanophore of *S. canaliculatus* and the effectiveness of the yohimbine and phentolamine to inhibit competitively the effect of the former demonstrate further, that, melanophores of this teleost, as generally the case is in other teleosts, are adrenergicaly innervated and the neuro-melanophore signals transduction is mediated through postsynaptic alpha adrenoceptors (Fujii and Oshima, 1994).

Melanophores in the winter flounder *Pseudopleuronectes americanus* show significant enhancement if subjected to repeated *in vitro* K^+ and Na^+ stimulation. It has been shown that three consecutive cycles of K^+ and Na^+ stimulation result in pronounced enhancement of K^+ evoked melanosome aggregation between the first and the second cycles and Na^+ evoked melanosome dispersion during the first three cycles (Burton and Everard, 1990). Also, *in vivo* experiments on this teleost involving repeated black and white background reversals and repeated injections of noradrenaline, have demonstrated similar enhancement (Burton and O'Driscall, 1992). Furthermore, it has been shown that administration of isotonic KCl induces melanosome aggregation in melanophores of guppy that could be maintained during repetitive application of the medium. However, no enhancement in the repeated response was observed. In contrast, repeated administration of isotonic KCl to melanophores of goby resulted in considerable decline in the degree of the response (Miyata and Yamada, 1987). On the other hand, in *Tilapia melanopleura*, no enhancement in the responsiveness of melanophores was observed, but instead fatigue was displayed after four consecutive K^+ stimulations (Custrucci, 1975).

Melanophores of *S. canaliculatus* did no show any sign of fatigue in their response to repeated (up to 20 stimuli) potassium stimulation. This clearly demonstrates that melanophore of *S. canaliculatus* is densely innervated and probably the remarkable rate of its melanosome motility is due to this dense innervation. Naturally, the prompt and fatigue free responses of the present tissue (30 sec in response to K^+ and 60 sec in response to noradrenaline) compared for example with the much slower responses of Pseudopleuronectes

americanus to potassium (12 min) and to norepinephrine (20 min), give the present tissue an advantage in studies related to the physiology and pharmacology of adrenergic synapse that require application of repeated chemical and/or electrical stimulations. However, intrinsic changes between the stimuli that may develop as a result of enhancement of responses must be taken in consideration (Burton and Everard, 1990; Burton and O'Driscall, 1992).

Postsynaptic alpha adrenoceptors in the mammalian tissues, based on their preferential affinity to specific adrenergic agonists have been subclassified into alpha-1 and alpha-2 receptors and adrenoceptors that show a high affinity toward clonidine and norepinephrine and much lees toward phenylephrine are of alpha-2 subtype (Starke *et al*., 1975). Using similar approach Andersson *et al*. (1984) were first to conclude that alpha adrenoceptors mediating melanosome aggregation in melanophores of the cuckoo wrasse, *Labrus ossifagus* are of alpha-2 subtype. Since then, melanosome aggregation within melanophores of several other teleostean fish has been subclassified as alpha-2 subtype (Karlsson *et al*., 1987; Morishita, 1987).

The responsiveness of *S. canaliculatus* melanophores is much higher to clonidine (specific alpha-2 adrenoceptor agonist) and norepinephrine (nonspecific adrenoceptor agonist) compared to phenylephrine and methoxamine (alpha-1 specific adrenoceptor agonists). In intact melanophores, the EC_{50} values obtained for clonidine (30 nM) and norepinephrine (50 nM) are much lower than that obtained for phenyephrine (300 nM) and methoxamine (950 nM). Furthermore, the failure of prazosine to inhibit melanosome aggregation induced by K^+-rich saline and adrenergic agonists in both intact and denervated melanophores, does not indicate any possible role of alpha-1 adrenoceptors in the mechanism of melanosome aggregation in this teleost. Phentolamine and yohimbine shifted the NE concentration-response curve to the right in the physiological concentration-dependent manner; their inhibiting potencies determined from shift values of EC_{50} are significantly different ($p<0.0001$). Yohimbine (alpha-2 specific) displayed a shift in values of EC_{50} from 50 to 2500 nM compared to the shift from 50 to 750 nM displayed by phentolamine (non-specific alpha antagonist). Thus, indicating a (3.3x) higher relative inhibiting potency of yohimbine. Therefore, the preferential affinity of the postsynaptic adrenoceptors mediating melanosome aggregation within the melanophores is clearly apparent to interact predominantly if not solely with alpha-2 specific agonists and antagonists.

The enhanced response of denervated melanophore to adrenergic agonists is well established (Burton and Vokey, 2000). In the cuckoo wrasse (Labrus ossifagus), denervated melanophores exhibited a 10^9 - fold increase in sensitivity to norepinephrine (Martensson and Andersson, 1997). The degree of the enhanced sensitivity of denervated melanophores that varies among different species of teleosts, has been explained in line with the hypersensitivity of the effector cells and tissues to catecholamines that usually follows peripheral denervation (Smith, 1941). Denervated melanophores of *S. canaliculatus* demonstrated similar enhanced responses to adrenergic agonists. Furthermore, in present preparation, the displayed enhancement in the responses of denervated melanophores to norepinephrine (76.9X) was found to be significantly greater than to their responses to clonidine (40X). As consequent, the rank order of relative potency in denervated melanophores changed to: NE (EC_{50} = 0.55 μM), clonidine (EC_{50} = 0.75 μM), phenylephrine (EC_{50}= 25 nM) and methoxamine (EC_{50} = 60 nM). This change in the rank order of relative potency in favor of norepinephrine is probably due to the absence of the neuronal re-uptake mechanism in denervated melanophores that is probably more specific to the reuptake of norepinephrine and hence resulting in the augmentation of its effect.

Karlsson *et al*. (1987) investigating responses of *L. ossifagus* melanophores to various adrenergic agents have reported that although the potency of phenylephrine to induce melanosome aggregation was only 1/1000 of that observed for the alpha-2 adrenoceptor selective agonists, but nevertheless it was able to function as a full agonist. The low potency of phenylephrine was speculated to be due to its very low affinity interaction with alpha-2 adrenoceptors. Furthermore, (Martensson and Andersson, 1997) studying on the same fish reported 10^9-fold increase in the sensitivity of denervated melanophores to both norepinephrine and melatonin. As the efficacy of melatonin increased from a negligible pigment-aggregation to the level of a full agonist that could be counteracted by yohimbine, but not by prazosine, it was concluded that the mediating receptors displaying post-denervation sensitivity is alpha-2 adrenoceptors. Similarly, in the present preparation, melanosome aggregation effects of phenylephrine and methoxamine (alpha-1 specific agonists) can be explained as a result of their interaction -although with a much lower affinity than clonidine and norepinephrine- with alpha-2 adrenoceptors. Also, the enhanced melanosome aggregation effects of phenylephrine and methoxamine in denervated preparations seems to be due to their more effective interaction with up-regulated receptors as a result of denervation.

Therefore, it is justifiable to speculate that melanosome aggregation in melanophores of *S. canaliculatus* is mediated through the stimulation of postsynaptic alpha-2 adrenoceptors. It is well known that the stimulation of alpha-2 adrenoceptors, have inhibiting effects on adenylate cyclase system and hence their activation will result in the depletion of the cytosolic level of cyclic AMP (Strake, 1981). It is also established that cyclic AMP function as a second messenger in the transduction of melanosome dispersion signals (Novales and Fujii, 1970; Fujii and Miyashita, 1976; Karlsson *et al*., 1985). Thus, the depletion in the level of cyclic AMP as a consequent effect of alpha-2 adrenoceptors stimulation induces melanosome aggregation within the melanophores.

In conclusion, the present study in addition of revealing the nature of mechanism involved in the melanosome aggregation in melanophores of *S. canaliculatus*, it also provides an excellent experimental tool that could be adopted in experiments designed to study the effects of candidate sympathomimetic and sympatholytic agents. Furthermore, as *S. canaliculatus* is a popular sea-food with high commercial value particularly in Arabian Gulf countries, understanding mechanisms involved in the regulation of its chromatic responses will contribute positively in the improvement of its aquaculture techniques.

ACKNOWLEDGMENTS

This research was supported by grant (slg/416/2005) from UAE University, Al-Ain, UAE. I am grateful to the Marine Resources Research Center, Um AL Quwain, Ministry of Environment and Agriculture, UAE, for the supply of experimental fish and to Ms. Emihan Tanedo for assistance with graphics study.

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