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Interaction of Some Nicotinoids and Biogenic Amines with Beta-adrenergic Receptors from Catfish Red Blood Cells

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Abstract. The *in vitro* interactions of some nicotinoids and biogenic amines with β_2-adrenergic receptor from catfish erythrocytes were studied. The results showed that:

- Nicotinoids are more potent effective than biogenic amines generaly at 100 uM.

- Nicotine was the strongest effective nicotinoid in this respect, it caused 65% inhibition, decreased the number of the maximum binding sites and increased the affinity of [^{3}H] DHA binding to the β_2-adrenergic receptor.

- Normetanephrine was the strongest effective biogenic amine, it caused 62.8% inhibition, has no effect on the number of the maximum binding sites and decreased the affinity of [^{3}H] DHA binding to the β_2-adrenergic receptor.

- Nicotine interacted competitively with β_2-adrenergic receptor from catfish red blood cells *in vitro*, but normetanephrine interacted non-competitively with such receptors.

Introduction

Effects of nicotine on the peripheral autonomic system have been studied for more than one hundred years [1, p 167-177]. The pharmacological properties of nicotine are very complex with stimulating as well as inhibiting actions on both the peripheral and the central nervous systems (CNS). A directly stimulating effect of nicotine on the peripheral adrenergic nerve terminals has also been reported. [2-4]. Nicotine acting at different levels in the adrenergic neuron may increase the activity of the neuron and the release of the transmitter noradrenaline (NA) [5]. In fish, the presence of alpha-and beta-adrenoceptors as defined by Ahlquist [6] has been demonstrated different tissues by the measurement of pharmacolgical responses to various catecholamines [7-10]. Adrenaline causes swelling of trout erythrocytes [11, p 103-119] which may be important in "stress" situations.

El-Sebae *et al.*, [12] used the equilibrium dialysis technique for studying the effect of some organophosphorus insecticides on the catecholamine β-receptor. The development of ligand binding techniques using a radiolabelled β-adrenergic antagonists [13] provides a direct approach for the characterization and quantification of β adrenoceptors, though such techniques have not been employed in vertebrates lower than the Amphibia. In radioligand binding studies using mammalian red blood cells the observed β-adrenoceptors have been condidered as belonging to the β_2-subtype. Beta-adrenoceptors in teleost erythrocytes don't belong to the β_1-subtype as classically defined, but in recent years many conclusions proved that this receptor is belonging to the β_2-subtype as further characterization using a wide range of specific agonists and antagonists. The close association between apposing adrenergic nand cholinergic axon terminals [14,15], has indicated that the situation is complex and probably includes a terminal interaction between the two nerve systems [16], whereby liberated acetylcholine influences not only the vascular smooth musculature, but also the adjacent adrenergic nerves at the sites of apposition.

Recently, β_2-adrenoceptors in the human heart have been found to couple to adenylate cyclase and to mediate positive inotropic and chronotropic actions [17, 18]. Since T-0509 ([(–)–(R)–1–(3,4 dihydroxyphenyl)–2–[(3,4-dimethoxyphenethyl) amino] ethonol]-hydrochloride) was a highly selective and full β_1-adrenoceptor agonist, it had weak β_2-adrenoceptor agonists and minimum α-adrenoceptor agonistic actions [19].

The present study was undertaken to investigate the *in vitro* interactions of nicotinoids and biogenic amines with beta 2-adrenergic receptor.

Materials and Methods

(–) Nicotine (98%), Nornicotine (98%), (–) Anabasine (99%), and DL-Conine-hydrochloride (98%) were obtained from Carl Roth OHG. Adrenaline, DL-octompamine, DL-Normetanephrine, DL-Metanephrine hydrochloride and Tyramine hydrochloride were obtained from Sigma Chemical Company, USA. These compounds were disolved in ethanol.

Membrane preparation of β-adrenergic receptor from catfish erythrocytes

All procedures were performed according to Bennett and Rankin [20]. Blood was taken from catfish by direct puncturing of the heart, using heparinized syringe. Continuous percoll$^{(R)}$ density gradients (Pharmacia Fine Chemicals), with a starting density of 1.08 g/ml; were self-formed at 20,000 xg for 60 min. Aliqouts of blood were layered over these gradients and centrifuged at 400 xg for 15 min to separate erythrocytes from other blood components. Red cells were aspirated off and washed three times with 5 volumes (original blood volume) of 120 mM NaCl; 10 mM Tris-HCl buf-

fer, pH 7.6, with intermediate sedimentation at 750 × for 5 min. Haemolysis was performed with 10 volumes of 5 mM Tris-HCl buffer, pH 7.8, followed by centrifugation at 20,000 xg for 15 min. This step was repeated three times. The final membrane pellet was resuspended in 75 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.8. The protein concentration was determined according to Lowery *et al.*, [21]. Final concentration of 100-150 ug protein / 100ul was obtained by dilution with the mentioned buffer.

Binding assay

Tritiated dihydroolprenolol ([^{3}H]DHA), specific activity 78 Ci/m mole, binding to plasma membrane was determined by rapid filtration technique [20]. Membrane protein 100-150 ug was incubated in a final volume of 300 ul containing 20 nM [^{3}H] DHA and Tris-HCl buffer pH 7.8. After incubation for 45 min., the membrane was filtered followed by rapid washing 3×5 ml of ice cold buffer. Filteration and washing required a total time less than 20 sec. The filterate was placed in 10 ml toluene scintillation spectrometer after 12 hr. Parallel incubations were carried out in each assay, with the presence of 20 uM DL-propranolol, to assess the nonspecific binding. The binding assay in the presence of nicotinoids and biogenic amines were done by add 100 μM of each them.

Results and Discussion

Competition of various nicotinoids and biogenic amines with [^{3}H] DHA binding to its specific binding sites

Effect of nicotinoids and biogenic amines on the specific [^{3}H] DHA binding to β_2-adrenergic receptor from catfish erythrocyte at 100 uM is presented in Fig. 1 and 2, respectively. It is clear that nicotine caused the highest percent of inhibition (65%), while nornicotine, anabasine, and coniine caused 55.7%, 51.7%, and 40.2% binding inhibition, respectively (Fig. 1). In Fig. 2 data showed that normetanephrine caused the highest percent of inbhibition (62.8%, while octapamine, metanephrine, adrenaline, and tyramine caused 40.7%, 38.4%, 36.6%, and 14% binding inhibition, respectivily. The previous data indicate that nicotine (nicotinoid) and normetanephrine (biogenic amines) were more potent effective than the other tested compounds.

Effect of nicotine and normetanephrine on the maximum binding sites and the dissociation constant of [^{3}H] DHA binding to The β_2-adrenergic receptor

The displacement of [^{3}H] DHA binding by nicotine and normetanephrine were shown by the common maximal [^{3}H] DHA binding in the absence (control) and presence of nicotine and normetanephrine in the scatchard plots, [22]. Scatchard analysis was designed to find out the nature of interaction of nicotine and normetanephrine with the DHA binding sites in the preparations of the catfish erythrocyte membrane.

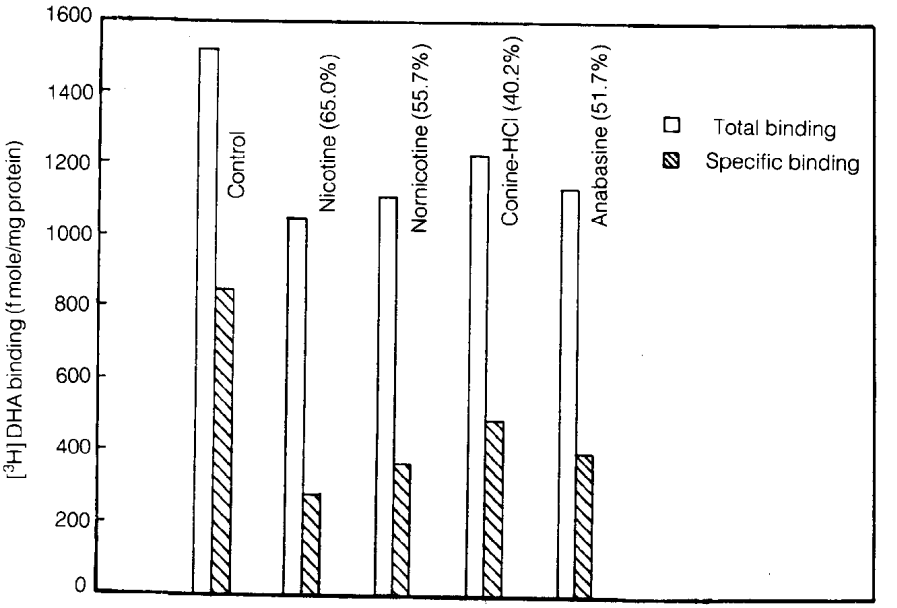

Fig. 1. Effect of some nicotinoids (100 μM) on the binding of [^{3}] DHA to catfish erythrocyte membranes β_2- adrenergic receptor. (as percent inhibition)

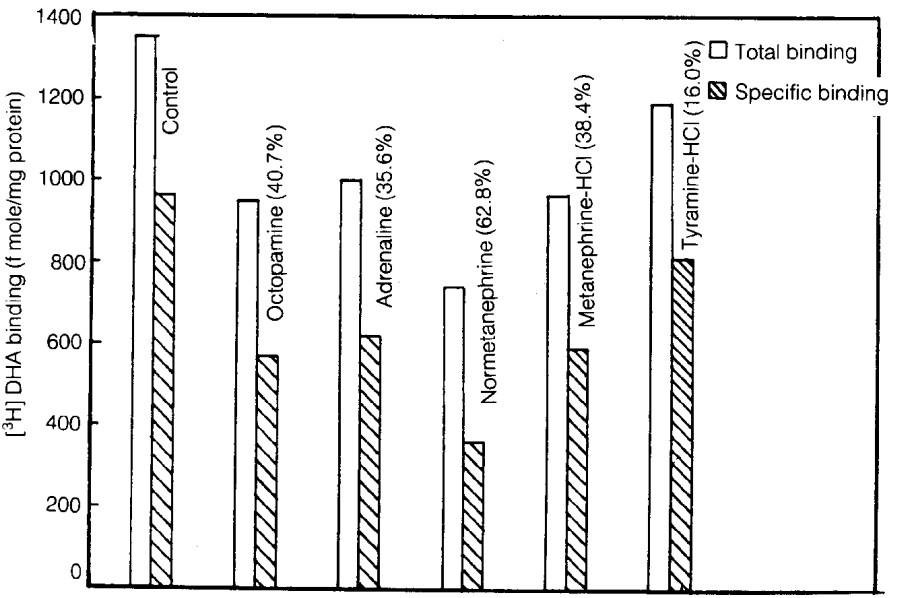

Fig. 2. Effect of some biogenic amines (100 μM) on the binding of [^{3}] DHA to catfish erythrocyte membranes β_2- adrenergic receptor. (as percent inhibition)

Figure 3 shows that nicotine decreased the number of the maximum binding sites "Bmax" from 450 fmol/mg protein in the absence of nicotine to 275 fmol/mg protein in presence of nicotine (100uM). Also the dissociation constant (K_a) has been decreased from 3.8 nM (in absence) of nicotine to 2.4 nM in its presence. These data indicate that nicotine increases the affinity of [^{3}H] DHA binding to the β_2-adrenergic receptor. In Fig. 4 the maximum binding sites "Bmax", for catfish erythrocyte membranes was 450 fmol/mg protein either in absence or in presence of normetanephrine (100 uM). The dissociation constant (K_a) incresed from 3.8 nM (in absence of normetanephrine to 6.3 nM in its presence. This means that the affinity of [^{3}H] DHA binding to the β_2-adrenergic receptor has been decreased.

The present study was undertaken to investigate the *in vitro* interaction of nicotinoids and biogenic amines with beta$_2$-adrenergic receptor. In general, the results indicated that nicotinoids were more potent effective than biogenic amines.

Recent studies have helped to elucidate many details regarding molecular architecture of the a β-adrenergic receptors [23, 24]. Results of these studies begin to provide more precise molecular identification of putative "efficacy sites" in the

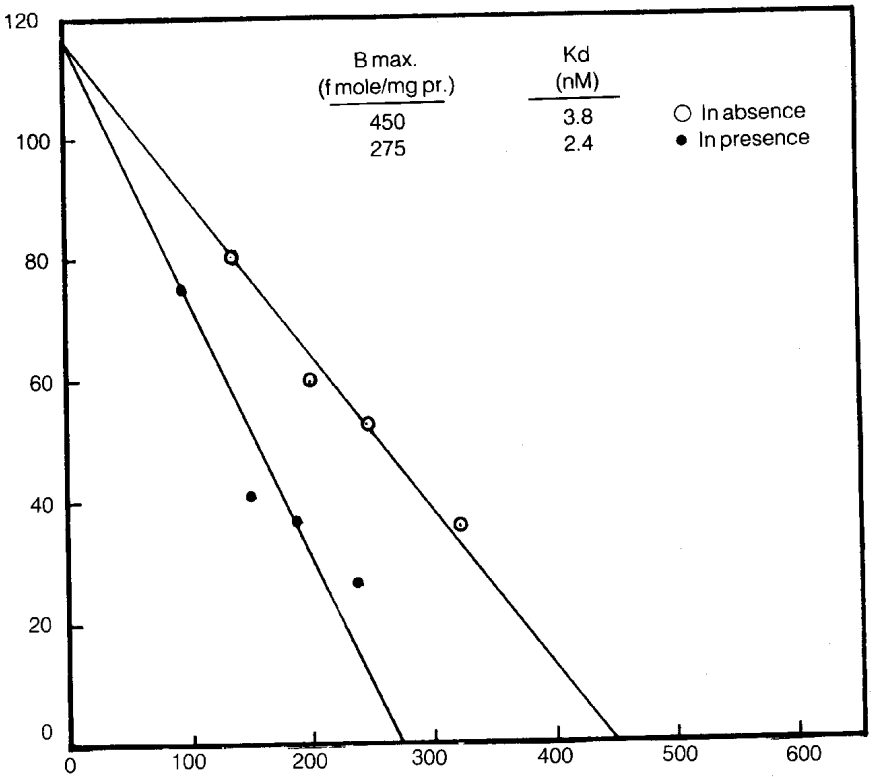

Fig. 3. Scatchard plots of [^{3}H] DHA binding in absence (0) and in the presence (•) of nicotine at 100 μM. (B/F = The ratio of specifically bound to free drug)

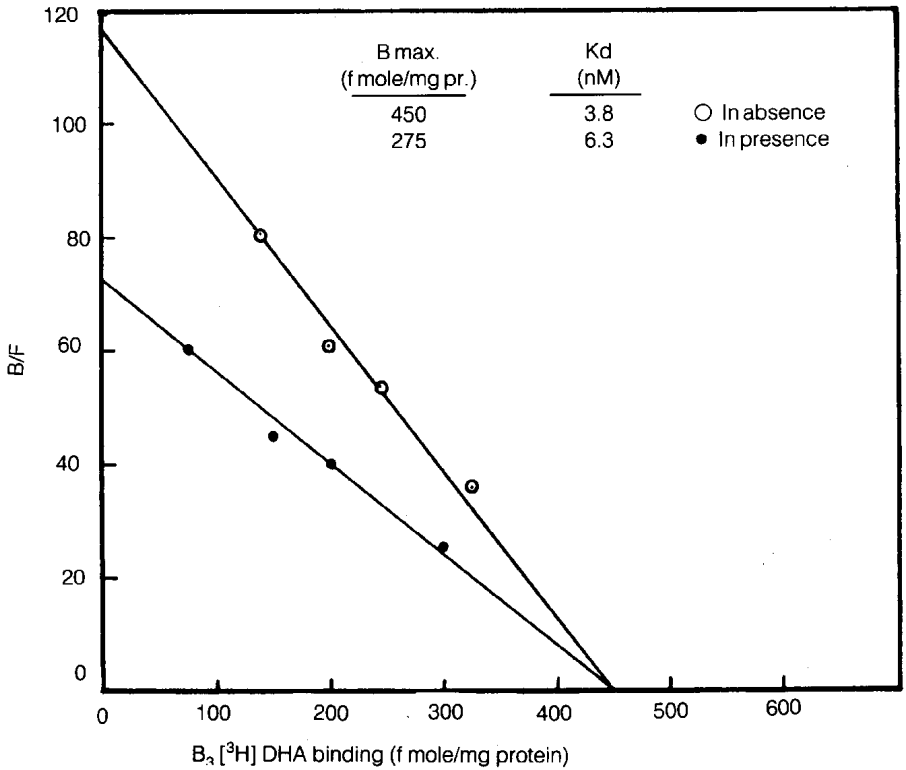

Fig. 4. Scatchard plots of [^{3}H] DHA binding in absence (0) and in the presence (•) of normetanephrine at 100 μM. (B/F = The ratio of specifically bound to free drug)

receptor. The predicted protein sequences of the β_1, β_2 and β_3 receptors are consistent with an extra cellular amino terminus, three cytoplasmic and extracellular loops, seven α-helical, hydrophobic plasma membrane spanning domains and an intra-cellular carboxy terminal tail [24] no need for this figure. Molecular-modelling of the binding of site β-adrenergic receptor suggests that ionic interaction between the protonated secondary amine of the ligand and the carboxylate side chain of Asp.^{113} deep in the receptor binding pocket may serve to initially orient the ligand [25].

At pH 7.0, i.e. near physiological pH, approximately 90% of nicotine in an aqueous environment exists in the protonated form ($P^ka = 7.9$) [26]. In vertebrates the effect of nicotine and its analogoues was attributed to the protonated form [27].

The intriguing result from this work is that nicotine may interact with an intra cellular carboxy terminal tail, therefore it decreases the maximum binding sites, (B_{max}). i.e. as an antagonist, it also increased the affinity for [^{3}H] DHA, i.e. as partial agent. These results agred with that of pindolol, where it was considered as an antagonist ligand, although it acted as partial against. [28].

We suggest that this phenomenon is due to the hydrogen-bonding of protonated nicotine with serine terminals of the receptor, which might induce conformational changes in its molecular structure.

On the other hand, normetanephrine decreased the affinity for [^{3}H] DHA binding to β_2-adrenergic receptor (2-fold), but had no effect on the maximum binding sites.

Studies on the structure - activity relationship of compounds were not adequate to predicte whether compounds will be partial agonists or in revealing the precise mechanism (s) for partial agonism. The substituents on the aromatic ring determine whether the compound activates or blocks the receptor [29]. For example, the catechol-hydroxy groups of isoproterenol, 3,4-dihydroxy-a-[isopropylamino) methyl] benzyl alcohol; a full agonist seems to be necessary for effective multipoint attachment with the β-receptor and maximal stimulation of the receptor [30]. Most competitive antagonists, typified by propranolol 1-(isopropylamino)-3-(1-naphthyl-oxy)-2-propanol lack these essential hydroxyl moieties and therefore do not stimulate the receptor.

In conclusion, nicotine interacts competitively with β_2-adrenergic receptor from atfish red blood cells *in vitro,* but normetanephrine interacts non-competitively.

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تداخل بعض أشباه النيكوتين والأمينات البيوجينية مع مستقبل بيتا ـ الأدرينالييني المفصول من كرات الدم الحمراء لقرموط السمك

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(قدم للنشر في ١٤١٤/٥/١١هـ؛ وقُبل للنشر في ١٤١٥/١/١٨هـ)

ملخص البحث. تم دراسة تداخل بعض أشباه النيكوتين والأمينات البيوجينية مع مستقبل البيتا ٢ ـ الأدرينالييني المفصول من كرات الدم الحمراء لسمك القرموط. وقد أظهرت الدراسة النقاط التالية:

ـ أن أشباه النيكوتين أكثر تأثيرًا من الأمينات البيوجينية بصفة عامة عند تركيز ١٠٠ ميكرومولر.

ـ كان النيكوتين أشد أشباه النيكوتين المختبرة تأثيرًا، حيث أحدث تثبيطًا مقداره ٦٥٪ وقلل كذلك من عدد مواقع ارتباط مادة ثنائي الهيدروالبرونيولول المعلمة (دي. اتش. أية المعلمة) بمستقبل بيتا ٢ ـ الأدرينالييني كما أدى أيضًا إلى زيادة قيمة قابلية الارتباط (Affinity) لمادة دي. اتش. اية المعلمة لمستقبل بيتا ٢ ـ الأدرينالييني.

ـ كان النورميتانيفرن أكثر الأمينات البيوجنية المختبرة تأثيرًا، حيث أحدث تثبيطًا قدره ٦٢,٨٪ ولم يحدث تغيير في عدد مواقع ارتباط مادة دي. اتش. أية المعلمة بمستقبل بيتا ٢ ـ الأدرينالييني ولكنه قلل قيمة قابلية الارتباط لمادة دي. اتش. أية المعلمة لمستقبل بيتا ٢ ـ الأدرينالييني.

ـ تداخل النيكوتين مع مستقبل بيتا ٢ ـ الأدرينالييني تنافسيًا بينما تداخل النورميتانيفرن مع المستقبل نفسه لا تنافسيًا.