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Postsynaptic α_1- and α_2-adrenoceptors in the vascular system of the herring gull, *Larus argentatus*

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1 The nature of the vascular α-adrenoceptors has been studied in the herring gull, *Larus argentatus*.

2 In the anaesthetized herring gull, both phenylephrine and clonidine elicited dose-dependent increases in arterial blood pressure.

3 The α_1-adrenoceptor antagonist prazosin was a better antagonist of phenylephrine than were the α_2-adrenoceptor antagonists yohimbine and rauwolscine.

4 Yohimbine and rauwolscine were better antagonists of clonidine than was prazosin.

5 The maximum response to phenylephrine, but not clonidine, was lower in reserpine-treated birds, indicating that phenylephrine in high doses liberates endogenous catecholamines, which contribute to the effect.

6 It is concluded that the herring gull possesses postsynaptic, vascular α-adrenoceptors, of both the α_1- and α_2-subtypes, similar to those found in mammals.

Introduction

The α-adrenoceptors have been shown to be a heterogeneous group (see reviews by e.g. Timmermans & Van Zwieten, 1981; Johansson, 1984). The dissimilarity of pre- and postjunctional α-adrenoceptors originally led Langer (1974) to identify the classical postsynaptic α-adrenoceptor as α_1 and the presynaptic α-adrenoceptor as α_2. The α_2-adrenoceptor has since been shown to be located also postsynaptically, and in vascular tissue, where this receptor type mediates vasoconstriction (Docherty *et al.*, 1979; Drew & Whiting, 1979; Docherty & McGrath 1980; Van Meel *et al.*, 1981).

Both α_1- and α_2-adrenoceptors can be activated by exogenously administered α-adrenoceptor agonists, which elicit an increase in blood pressure due to an increase in total peripheral resistance, caused by contraction of vascular smooth muscle.

These studies have mostly been performed in mammals, whereas little is known about receptor types in other animal classes (see Johansson, 1984). In this paper we have studied postsynaptic α-adrenoceptors in the vascular system of an avian species, the herring gull, *Larus argentatus*.

Some preliminary results were presented at the 5th Catecholamine symposium, Göteborg, 1983 (Hermansson & Johansson, 1983).

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Methods

Wild captured herring gulls, *Larus argentatus*, (0.6–1.2 kg body wt), were housed in outdoor cages and deprived of food for one day before experimentation, but had water accessible *ad libitum*.

Preparation

The animals were anaesthetized by intravenous administration of phenobarbitone (Fenemal, ACO, Sweden). An incision was made in the neck and the trachea was cannulated for artificial respiration. One of the carotid arteries was cannulated with a heparinized, saline-filled polyethylene catheter (PE 60) connected to a Statham p 23 Ac pressure transducer for continuous recording of arterial blood pressure on a GRASS Model 7 Polygraph. An indwelling cannula, attached to a catheter (PE 50), was inserted into the femoral vein for drug administration. The body temperature was maintained at 40 ± 0.5°C by means of an infrared lamp.

Hexamethonium bromide (20 mg kg^{-1}, i.m.) and atropine (25 mg kg^{-1}, i.v.) were given about 0.5 h before the experiment to abolish reflex activity.

One group of animals was pretreated with reserpine 5 mg kg^{-1}, i.m. 15–20 h before the experiments. In addition, 1.25 mg kg^{-1}, i.m. was given 1 h before the experiments.

Experimental procedure

When the blood pressure has stabilized, single doses of increasing concentration of an agonist were given (0.1 ml kg^{-1}, i.v.) followed by 0.5 ml saline, until a maximum pressure response was obtained. Only one or two individual dose-response curves were obtained from each animal. In the case of two curves, the first was a control, followed by a second with an antagonist present or, in a few birds, another control curve. There was no statistical difference between the first and second curve obtained from one bird. The blood pressure was allowed to return to the basal level between the injections. When agonist/antagonist interaction was studied, the antagonist was given as a single dose 15 min before injection of the first dose of agonist. Log dose-response curves were constructed, and the response was expressed as increase in mean arterial blood pressure (mmHg).

Statistics

The results are expressed as mean values ± s.e.mean. Since the number of experiments was relatively small, the non-parametric Mann-Whitney U-test was used (Siegel 1956). Significant differences were established at the $P < 0.05$ level.

Drugs

The following drugs were used: atropine sulphate (Sigma); clonidine HCl (Boehringer-Ingelheim); hexamethonium bromide (Sigma); (−)-phenylephrine HCl (Sigma); prazosin HCl (Pfizer); rauwolscine HCl (Roth); reserpine (Serpasil, 2.5 mg ml^{-1}; Ciba) and yohimbine HCl (Sigma).

Prazosin was dissolved in a few drops of glacial acetic acid and diluted with 5.5% glucose. Yohimbine and rauwolscine were dissolved in a small amount (a few drops) of ethanol and diluted with 5.5% glucose and saline, respectively. Doses refer to the forms indicated above.

Results

Blood pressure responses

In anaesthetized herring gulls, the mean arterial blood pressure (MAP) was 86 ± 2 mmHg, $n = 72$. After pretreatment with hexamethonium and atropine, the resting blood pressure was found to be 85 ± 3 mmHg, $n = 72$. The heart rate was not measured (except in a few birds to establish the effectiveness of atropine and hexamethonium in blocking reflexogenic activity). The α-adrenoceptor agonists (−)-phenylephrine and clonidine elicited dose-dependent increases in MAP (Figure 1). However, the maximum response to phenylephrine was about twice that to clonidine. (Higher doses of phenylephrine than indicated in Figure 1 were tested only in a few birds since these doses clearly had toxic effects, inducing arrhythmias and thus a marked fall in blood pressure.)

The negative logarithm of the dose of agonist (mol kg^{-1}) increasing the blood pressure to 50% of the maximum response (pD_2) was 7.38 ± 0.07 for phenylephrine ($n = 6$) and 8.05 ± 0.20 for clonidine ($n = 6$).

Reserpinized birds The resting MAP in anaesthetized, reserpinized birds was 75 ± 2 mmHg, $n = 69$. After an additional dose of reserpine (1.25 mg kg^{-1}), and hexamethonium and atropine, the blood pressure was found to be 72 ± 3 mmHg, $n = 69$. Following this treatment, there was no significant change in pD_2 (phenylephrine; 7.57 ± 0.06, $n = 6$; clonidine; 7.77 ± 0.11, $n = 6$). However, the response to the highest dose of phenylephrine was smaller (Figure 1).

Interaction with antagonists

Pretreatment with the α_1-selective antagonist prazosin, 10^{-7}, 10^{-6} or 10^{-5} mol kg^{-1} caused a dose-dependent rightward shift of the log dose-response curve for phenylephrine (Figure 2, upper panel) but not the clonidine dose-response curve (Figure 3, upper panel). Prazosin did not affect the blood pressure or heart rate

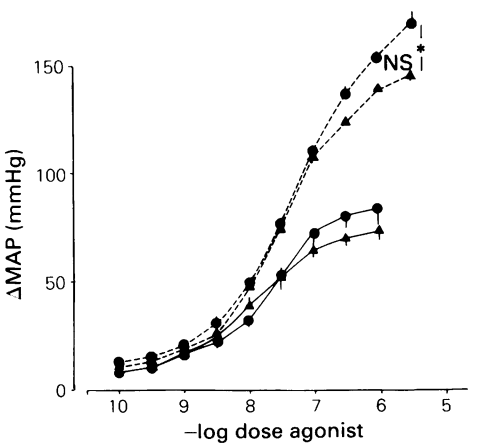

Figure 1 Log dose-response curves for (−)-phenylephrine and clonidine for the increase in mean arterial blood pressure (MAP) in reserpine-treated and untreated, anaesthetized, ganglion-blocked and atropinized herring gulls. Solid lines = clonidine; dashed lines = phenylephrine; unreserpinized (●); reserpine pretreated (▲). Vertical bars indicate s.e.mean, $n = 6$; NS = not significant. $^*P < 0.05$.

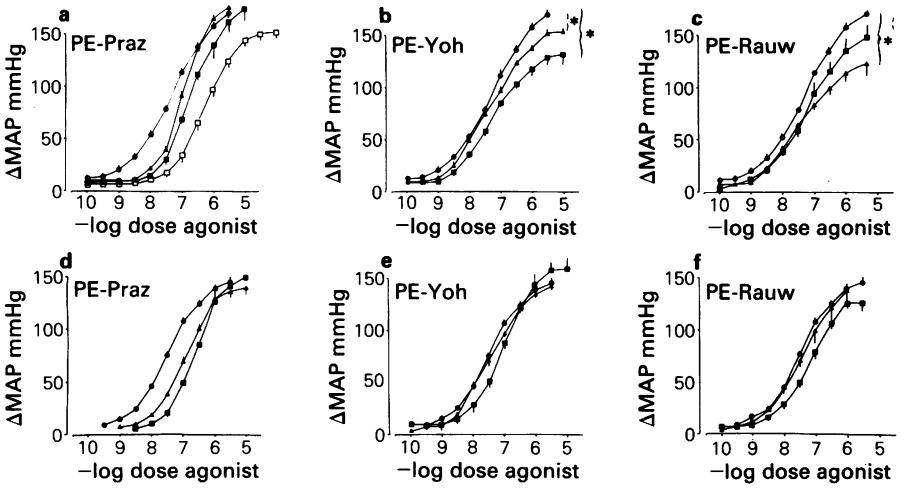

Figure 2 Log dose-response curves for the increase in mean arterial blood pressure (MAP) in anaesthetized, ganglion-blocked and atropinized herring gulls. Effects of antagonists on pressor responses to i.v. phenylephrine (PE). Upper panels: non-reserpinized. Lower panels: reserpine pretreated. (a) and (d) Prazosin (Praz); (b), (e) yohimbine (Yoh); (c), (f) rauwolscine (Rauw). (●) Control; (▲) antagonist 10^{-7} mol kg^{-1}; (■) antagonist 10^{-6} mol kg^{-1}; (□) antagonist 10^{-5} mol kg^{-1}. *Significant ($P<0.05$) differences between control and antagonist-treated curves for maximum responses. Vertical bars indicate s.e.mean, $n = 4-7$.

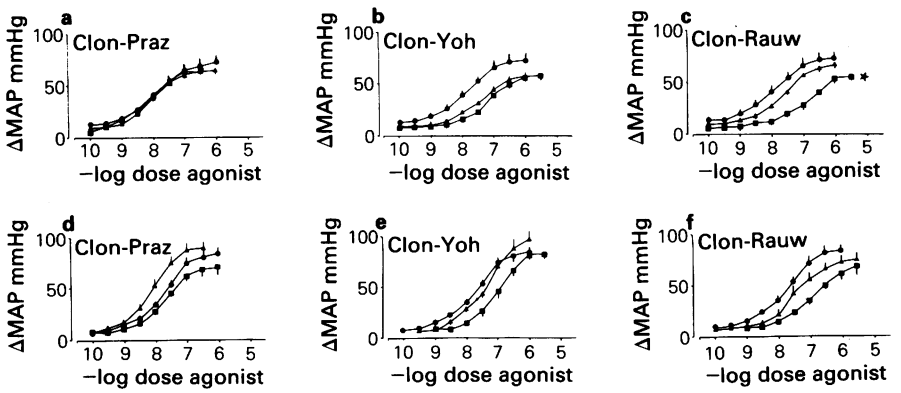

Figure 3 Log dose-response curves for the increase in mean arterial blood pressure (MAP) in anaesthetized, ganglion-blocked and atropinized herring gulls. Effects of antagonists on pressor responses to i.v. clonidine (Clon). Upper panels: non-reserpinized. Lower panels: reserpine pretreated. (a), (d) Prazosin (Praz); (b), (e) yohimbine (Yoh); (c), (f) rauwolscine (Rauw). (●) Control; (▲) antagonist 10^{-7} mol kg^{-1}; (■) antagonist 10^{-6} mol kg^{-1}. *Significant ($P<0.05$) differences between control and rauwolscine 10^{-6} mol kg^{-1} for maximum responses. Vertical bars indicate s.e.mean, $n = 4-7$.

significantly *per se*. The α_2-selective antagonists yohimbine, 10^{-7}, 10^{-6} or rauwolscine 10^{-7} or 10^{-6} mol kg^{-1}, caused dose-dependent rightward shifts of the clonidine dose-response curve (Figure 3, upper panel). These antagonists also shifted the dose-response curve for phenylephrine to the right, but to a lesser degree (Figure 2, upper panel). The rightward shift was dose-dependent for yohimbine but not for rauwolscine. The maximum obtainable increase in MAP to phenylephrine was significantly ($P<0.05$) decreased by either yohimbine or rauwolscine (except rauwolscine 10^{-6} mol kg^{-1}). The maximum increase in MAP to clonidine was not significantly affected by any of the antagonists, except after rauwolscine 10^{-6} mol kg^{-1}, ($P<0.05$). Neither yohimbine nor rauwolscine affected significantly the resting blood pressure or heart rate.

Reserpinized birds In a separate series of experiments, the birds were treated with reserpine.

Prazosin, yohimbine or rauwolscine (10^{-7}–10^{-6} mol kg^{-1}) caused dose-dependent rightward shifts of the phenylephrine log dose-response curves (Figure 2, lower panel). Prazosin was more effective than yohimbine and rauwolscine in this respect. The response to the largest dose of phenylephrine was significantly ($P<0.05$) smaller after reserpine treatment (Figure 1).

The effect of clonidine in reserpinized birds after prazosin pretreatment varied with the dose. The low dose of prazosin (10^{-7} mol kg^{-1}) shifted the mean clonidine dose-response curve to the left, while the high dose (10^{-6} mol kg^{-1}) gave the expected rightward shift (Figure 3, lower panel).

Both yohimbine and rauwolscine (10^{-7}–10^{-6} mol kg^{-1}) shifted the clonidine dose-response curve to the right in a dose-dependent way. The maximum response was not significantly affected.

Discussion

The studies leading to subdivision of α-adrenoceptors have mainly been performed in mammals, whereas little is known about adrenoceptors in other animal classes. The presence of α- and β-adrenoceptors in the vascular smooth muscle of the domestic fowl has been demonstrated by Bolton & Bowman (1969). However, the nature of the vascular α-adrenoceptors has not yet been described in birds. The meagre information regarding α-adrenoceptor classification in birds thus prompted an investigation of the nature of these receptors in birds' vascular tissue.

Wild captured herring gulls were used throughout this study. They have been used in previous studies regarding cardiovascular control, and proved to be suitable for this purpose (Johansson *et al.*, 1981; 1983a,b). Since the animals used were caught in their wild habitat, there may be seasonal as well as individual variations influencing the results. In some of our results, oddities may derive from such variations. An *in vivo* model was used since a few experiments on large isolated arteries indicated only α-adrenoceptors of the α_1-subtype (data not presented).

It is obvious that antagonists disappear from the circulation in the kind of preparation used here, which is reflected as a deviation from parallelism in the dose-response curves. For this reason and the lack of an equilibrium response to agonists, no reliable pA_2-values could be calculated.

In mammals, the postsynaptic α-adrenoceptors, both the α_1- and the α_2-subtype, mediate pressor responses due to contraction of vascular smooth muscle (Docherty & McGrath, 1980; Timmermans *et al.*, 1980; Timmermans & Van Zwieten, 1981). The increase in blood pressure, following activation of α-adrenoceptors often causes a reflex increase in vagal activity and a decrease in sympathetic activity (see e.g. reviews by Korner, 1971; Korner & Angus, 1981) and thus decreases the measured pressor response. This has also been observed in birds (Durfee, 1964). Therefore reflex activity was abolished by administration of the ganglion blocking agent, hexamethonium, and the muscarinic receptor antagonist, atropine. After this treatment, no compensatory baroreflex was observed when tested by means of i.v. injections of (−)-phenylephrine (compared with the effect of phenylephrine in intact birds, where the increase in blood pressure is paralleled by a marked reduction in heart rate). The effectiveness of the ganglion blockade and atropinization is further demonstrated by the lack of centrally induced reductions in blood pressure or heart rate, which normally follow i.v. injections of clonidine in the herring gull (Johansson *et al.*, 1981).

(−)-Phenylephrine was selected as an α_1-agonist and clonidine as an α_2-agonist, since these drugs are known to be fairly selective in mammals (Starke *et al.*, 1975; Wikberg, 1978), although they each activate both types at high doses (Docherty & McGrath, 1980; Flavahan & McGrath, 1980). In the herring gull, pD_2 values for phenylephrine and clonidine were found to be essentially the same. However, the largest response elicited by phenylephrine (hereafter referred to as 'maximum') was twice as large as the maximum to clonidine. Some possibilities are that there is a denser population of α_1-adrenoceptors in the vascular system, thus eliciting a greater response, or that α_1-adrenoceptors are located in more vessels. The absolute size of the maximum pressor response to clonidine is in accordance with the findings from α_2-stimulation in the vagotomized, ganglion blocked rabbit (Van Meel *et al.*, 1982), but the largest response to α_1-stimulation was much higher in the herring gull than in that study of the rabbit. The onset of the

pressor response to phenylephrine was very rapid, compared to clonidine. This condition was also found in rats by Van Meel and co-workers (1982).

Depletion of catecholamines by use of reserpine, was carried out to discover whether the agonists used act via release of endogenous catecholamines. Depletion of catecholamines is known to increase the sensitivity of the effector tissues under certain conditions (Hudgins & Fleming, 1966; Carrier & Shibata, 1977). However, no such phenomenon was seen in the herring gull; on the contrary, the 'maximum' response to phenylephrine was significantly lower in the reserpine-treated birds. This may indicate that phenylephrine in high doses liberates endogenous catecholamines, a property of phenylephrine which is well known from mammals (see e.g. Innes & Nickerson, 1975). In birds not treated with reserpine, selective antagonism of α_2-adrenoceptors but not of α_1-adrenoceptors, decreased the 'maximum' response to phenylephrine to a similar degree, as did reserpine, whereas in reserpinized animals, no such reduction was seen. This contributes to the speculation that in unreserpinized birds, catecholamines liberated by phenylephrine contribute to the maximum effect by activation of α_2-adrenoceptors. The possibility that phenylephrine liberates endogenous catecholamines is further supported by the finding that the highest doses of phenylephrine also increased the heart rate slightly. This effect was diminished by administration of propranolol or reserpine but not prazosin, which indicates involvement of β-adrenoceptors, probably activated by endogenous catecholamines (data not presented). Clonidine, on the other hand, showed no ability to release endogenous catecholamines, or directly influence heart rate, as judged by the lack of effect of reserpine on the pressor response and clonidine's lack of effect on heart rate in either reserpine-treated or untreated birds.

Prazosin is known to be a highly selective α_1-antagonist in mammals (Cambridge *et al.*, 1977; Davey, 1980). In the dose range used in this study, it gave a rightward shift of the dose-response curve for phenylephrine in unreserpinized birds. The α_2-selective antagonist yohimbine (Timmermans *et al.*, 1980; Starke, 1981) also shifted the phenylephrine dose-response curve to the right in a dose-dependent way, but in equimolar doses, the response was less pronounced than for prazosin. Rauwolscine, which in mammals is a highly selective α_2-adrenoceptor antagonist (Timmermans *et al.*, 1980; Starke, 1981), also produced a rightward shift of the phenylephrine dose-response curve, which, however, was not proportional to dose.

Clonidine, on the other hand was markedly antagonized by yohimbine and rauwolscine in a dose-dependent manner, while no shift was obtained with prazosin.

The pressor response to clonidine was markedly antagonized by yohimbine and rauwolscine in a dose-dependent manner in reserpinized birds, whereas prazosin (10^{-7} mol kg^{-1}) caused a leftward shift of the clonidine dose-response curve. However, a higher dose (10^{-6} mol kg^{-1}) slightly shifted the curve to the right. The reason for the left shift is not clear but it cannot be excluded that this effect is derived from natural variation among herring gulls.

For yohimbine and rauwolscine, the dose-response curve for clonidine was dose-dependently shifted to the right in reserpinized birds, and the rightward shifts were more pronounced than for prazosin in equimolar doses.

In reserpinized birds, prazosin showed a clear preference for the phenylephrine-activated receptor, while yohimbine and rauwolscine showed selectivity for the clonidine-activated receptor. These results are very similar to findings in mammals (Timmermans & Van Zwieten, 1981) and thus indicate the presence of both α_1- and α_2-adrenoceptors.

In conclusion, our results show that phenylephrine and clonidine increase blood pressure in ganglion-blocked, atropinized herring gulls by acting on post-synaptic α-adrenoceptors. The maximum increase in MAP to phenylephrine is about twice as large as that to clonidine. It is also indicated that phenylephrine releases endogenous catecholamines, which may contribute to the effect, whereas no such property was observed for clonidine. The α_1-adrenoceptor agonist phenylephrine is markedly antagonized, in a dose-dependent way, by the α_1-antagonist prazosin, and to a lesser degree by the α_2-antagonists yohimbine and rauwolscine. The α_2-adrenoceptor agonist clonidine is markedly antagonized by yohimbine and rauwolscine, but not by prazosin. These findings strongly indicate the presence of vascular, postsynaptic α-adrenoceptors of both the α_1- and α_2-subtype, similar to those found in mammals.

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