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[^{3}H]Dihydroergonine Binding to α-Adrenergic Receptors in Human Platelets

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Bindung von [^{3}H]Dihydroergonin an α-adrenerge Rezeptoren in menschlichen Thrombozyten

Zusammenfassung. Die Bindung von [^{3}H]Dihydroergonin, einem potenten α-adrenergen Blocker in menschlichen Thrombozyten, wurde in intakten menschlichen Thrombozyten und Thrombozyten-Membranen untersucht. Die Bindung erreichte ihr Äquilibrium innerhalb von 10 min bei 25° C und war reversibel nach Zugabe eines Überschusses von Phentolamin. Die Geschwindigkeitskonstanten betrugen 0,31 min^{-1} für die Vorwärtsreaktion und 0,027 min^{-1} für die Rückwärtsreaktion, woraus eine Assoziationsgeschwindigkeitskonstante von $2{,}2 \times 10^7$ M^{-1} min^{-1} berechnet wurde. Die [^{3}H]Dihydroergonin-Bindung erreichte Sättigung mit 220 fmol [^{3}H]Dihydroergonin gebunden pro mg Membran-Protein und etwa 200 Bindungsstellen pro Thrombozyt. Äquilibriums-Untersuchungen ergaben eine einheitliche Population von Bindungsstellen ohne Anzeichen einer Kooperativität und eine Dissoziations-Konstante von 6 bis 7 nM. Die Bindung von [^{3}H]Dihydroergonin zeigte alle typischen Charakteristika der Bindung an einen α-adrenergen Rezeptor and Stereoselektivität: Adrenerge Agonisten bzw. Antagonisten konkurrierten um die Bindungsstellen in der Potenzreihe: (1)-Adrenalin > (1)-Noradrenalin > (d)-Noradrenalin ≫ (1)-Isoprenalin bzw. Yohimbin > Dihydroergotamin > Phentolamin ≫ Tolazolin > Azapetin ≫ (1)-Propranolol > (1)-Pindolol.

Es wurde versucht, die Bindungsdaten verschiedener α-adrenerger Agonisten mit der Thrombozyten-Aggregation und der Hemmung der Adenylat-Cyclase zu korrelieren. Unter verschiedenen getesteten α-adrenergen Agonisten waren nur Adrenalin und Noradrenalin fähig, eine primäre Thrombozyten-Aggregation auszulösen und die Adenylat-Cyclase zu hemmen. Verschiedene andere Substanzen, die in anderen Systemen α-adrenerge Agonisten sind, verdrängten [^{3}H)Dihydroergonin von seinen Bindungsstellen mit etwas geringeren Affinitäten als Adrenalin, so Phenylephrin und Methoxamin, oder mit weit höheren Affinitäten, so die Imidazoline Xylometazolin, Oxymetazolin, Naphazolin und Tetryzolin. Im Gegensatz zu Adrenalin und Noradrenalin waren diese Substanzen aber nicht fähig, eine primäre Thrombozyten-Aggregation (bis 1 mM) und eine Hemmung der Adenylat-Cyclase (bis 100 μM) zu induzieren. Die Ergebnisse zeigen, daß der thrombozytäre α-adrenerge Rezeptor sich nicht wesentlich von α-adrenergen Rezeptoren in anderen Systemen unterscheidet, was die Bindung von Substanzen an den Rezeptor angeht, daß aber nur ein limitiertes Spektrum von Substanzen fähig ist, über diesen Rezeptor biologische Antworten hervorzurufen.

Schlüsselwörter: α-adrenerge Rezeptoren — Thrombozyten-Aggregation — Thrombozytäre Adenylat-Cyclase — α-adrenerge Wirkungen.

Summary. Binding of [^{3}H]dihydroergonine, a potent α-adrenergic blocking agent, was studied in intact human platelets and platelet membranes. The binding process reached equilibrium within 10 min at 25° C and was reversible upon addition of excess phentolamine with forward and reverse rate constants of 0.31 and 0.027 min^{-1}, respectively, and with a second order association rate constant of 2.2×10^7 M^{-1} min^{-1}. The [^{3}H]dihydroergonine binding reached saturation with 220 fmol of [^{3}H]dihydroergonine bound per mg of platelet membrane protein and about 200 binding sites per platelet. Equilibrium studies indicated a single population of binding sites with no apparent cooperativity and a dissociation constant of 6 to 7 nM. Binding of [^{3}H]dihydroergonine showed all the

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typical characteristics of binding to an α-adrenergic receptor and stereoselectivity: Adrenergic agonists and antagonists competed for the binding sites with the following order of potency: (1)-adrenaline > (1) - noradrenaline > (d) - noradrenaline ≫ (1) - isoprenaline and yohimbine > dihydroergotamine > phentolamine ≫ tolazoline > azapetine ≫ (1)-propranolol > (1)-pindolol, respectively.

It was tried to correlate the binding data of various α-adrenergic agonists with platelet aggregation and inhibition of adenylate cyclase. Out of many α-adrenergic agonists tested, only adrenaline and noradrenaline were able to induce primary platelet aggregation and inhibition of adenylate cyclase. Various other compounds that are α-adrenergic agonists in other systems displaced [^{3}H]dihydroergonine from its binding sites with somewhat lower affinities than adrenaline, e.g., phenylephrine and methoxamine, or with much higher affinities, e.g., the imidazolines, xylometazoline, oxymetazoline, naphazoline and tetryzolin. In contrast to adrenaline and noradrenaline, these compounds were unable to induce primary platelet aggregation (up to 1 mM) and inhibition of adenylate cyclase (up to 100 μM). These data indicate that the platelet α-adrenergic receptor does not greatly differ from α-adrenergic receptors found in other tissues with respect to binding of drugs to the receptor but that the platelet receptor is unique with regard to the limited spectrum of compounds that are capable of inducing biological responses.

Key words: α-adrenergic receptors — platelet aggregation — platelet adenylate cyclase — α-adrenergic responses.

Adrenaline and noradrenaline induce aggregation of human platelets [4, 18, 20, 21], cause a rapid decrease in prostaglandin E_1-enhanced cyclic AMP levels in intact platelets [6, 9, 10, 17, 22, 23] and induce immediate inhibition of platelet adenylate cyclase in cell-free preparations [12, 13, 14]. The findings that these responses are blocked by α-adrenergic blocking agents such as dihydroergotamine and phentolamine, but not by β-adrenergic blocking agents, indicate that these adrenergic effects are mediated by α-adrenergic receptors.

Recently, [^{3}H]dihydroergocryptine, an α-adrenergic antagonist, has been shown to bind specifically to α-adrenergic receptors in different tissues of various species [7, 24, 25, 26] including human platelets [1, 15, 19]. We have recently shown that out of many α-adrenergic agonists tested only adrenaline and noradrenaline are able to induce primary platelet aggregation and inhibition of adenylate cyclase (14). This narrow spectrum of agonists, which is up to now found only in platelets, stimulated us to study the binding characteristics of the α-adrenergic receptor in human platelets, with special interest on α-adrenergic agonists.

In the present study, we report on the identification and characterization of α-adrenergic receptors in intact human platelets and platelet membranes with [^{3}H]dihydroergonine as labelled ligand and on the effects of various adrenergic agonists and antagonists on [^{3}H]dihydroergonine binding. This study indicates that various agents, which act as α-adrenergic agonists in other systems and which are without any apparent intrinsic activity in platelets with respect to aggregation and inhibition of adenylate cyclase, compete for [^{3}H]dihydroergonine binding sites with similar or even higher affinities than adrenaline and noradrenaline.

Materials and Methods

a) Reagents

[^{3}H]Dihydroergonine hydrochloride (26.8 Ci/mmol, tritiated at position 13 of the dihydrolysergic acid residue, Fig. 1), unlabelled dihydroergonine hydrochloride, dihydroergotamine methansulfonate and (1)-pindolol hydrochloride were obtained from Sandoz AG, Basel. The bitartrates of (1)-adrenaline and (1)-noradrenaline and the hydrochlorides of (1)-phenylephrine and yohimbine were purchased from Sigma, München. (1)-Isoprenaline bitartrate was donated by Boehringer Ingelheim, (1)-propranolol hydrochloride by ICI-Pharma, Plankstadt, phentolamine methansulfonate, naphazoline nitrate and the hydrochlorides of xylometazoline and tolazoline were obtained from CIBA-Geigy, Basel, (d)-noradrenaline bitartrate from Adams Chemicals, Chicago, (d,1)-methoxamine hydrochloride from Wellcome, Großburgwedel, oxymetazoline hydrochloride from E. Merck, Darmstadt, and tetryzolin hydrochloride from Pfizer, Karlsruhe. Aqueous solutions of adrenergic agonists and antagonists were prepared shortly before experiments. Ethanol, which was necessary for dissolving the ergot alkaloids, did not affect the binding at the highest concentration used (0.5%).

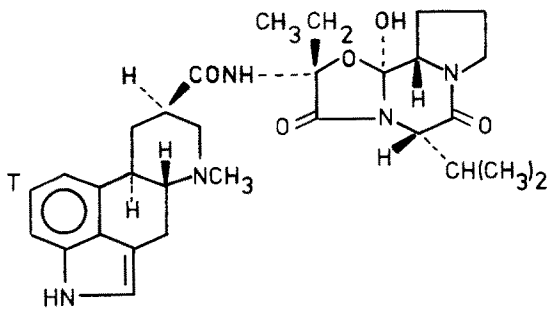

Fig. 1. Structure of [^{3}H]dihydroergonine. T marks the position of the tritium atom