NICOTINIC AGONISTS RELEASE SEROTONIN FROM RAPHEHIPPOCAMPAL TERMINALS

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It is widely known that nicotinic agents enhance transmitter release in various brain regions via neuronal nicotinic receptors. The altered brain serotonergic function is of great importance in psychiatric disorders. In the present study we investigated the effect of different nicotinic agonists (dimethylphenylpiperazinium-iodide, DMPP, nicotine, cytisine, (-)lobeline, and (-)epibatidine) and antagonists (mecamylamine and dyhidro-ß-erythroidine) on the release of [^{3}H]5-HT from hippocampal slices. The nicotinic agonists DMPP, lobeline, and electrical field stimulation released [^{3}H]5-HT from the hippocampus; other nicotinic agonists, such as nicotine, cytisine, and epibatidine, had no effect. The action of DMPP and lobeline was $[Ca^{2+}]_O$-independent and tetrodotoxin-insensitive. In Ca^{2+}-free medium DMPP was still able to release tritium from the hippocampus, while the stimulation-evoked release of [^{3}H]5-HT was abolished. Ca^{2+} channel blockers (cadmium, ω-conotoxin GVIA and nifedipine) could not modulate the effect of DMPP. Because of the dual character of the action of DMPP and lobeline, we investigated the effect of an ion channel modulator, flufenamic acid (FFA), on the release of [^{3}H]5-HT. FFA, at a concentration of 100 µM, inhibited the effect of DMPP and lobeline to release 5-HT from hippocampal slices. Therefore, it is proposed that the effect of DMPP and lobeline to enhance the release of [^{3}H]5-HT from the hippocampus was most likely mediated via nAChRs in part, while another way for DMPP to release 5-HT may be the modulation of ion channels.

DOWN REGULATION OF MUSCARINIC RECEPTORS INDUCED BY SODIUM NITROPRUSSIDE

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In the present study we have extensively characterized the down regulation of muscarinic acethylcholine receptors induced by the nitrogen monoxide (NO) generating compound sodium nitroprusside (SNP). When CHO cells stably transfected with the m4 muscarinic receptor subtype were incubated for 1 h in the presence of 700 µM SNP, the number of receptors, measured in intact cells with the hydrophilic ligand [^{3}H]N-methylscopolamine ([^{3}H]NMS), was reduced by 30 %. This effect was dose dependent, beginning with concentration of SNP as low as 45 µM. The time course of receptor desensitization induced by SNP was very fast ($t_{1/2}$ ranged between 10 and 20 min). Removal of SNP from the incubation medium did not result in a recovery of the binding sites measured with [^{3}H]NMS. The phenomenon was temperature dependent (it did not occur at 4 °C), and was blocked by the muscarinic antagonist atropine. Moreover, the effect of SNP was not observed in cell homogenate indicating that the integrity of the cell was required. Receptor diminution was not detected when the number of binding sites was evaluated with the lipophilic antagonist [3H]quinuclidynil benzilate. This indicates that SNP induces a redistribution of the muscarinic receptors between the plasma membrane and an internal compartment of the cell. Receptor loss was readily reversed by the treatment with the sulphydryl reducing agent diethyldithiocarbamate (10 µM). Our data provide evidence that muscarinic receptors are down regulated by SNP through the oxidation of sulphydryl groups; moreover they suggest that NO could play a role in muscarinic receptor desensitization.

EFFECTS OF BENZYDAMINE ON MOUSE MUSCLE TYPE NICOTINIC ACETYLCHOLINE RECEPTOR EXPRESSED IN *XENOPUS* OOCYTES.

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Benzydamine is a topically administered nonsteroidal antiinflammatory drug (NSAID) endowed also with a local anesthetic activity. Many local anesthetics and their derivatives block electrically excitable Na^+-channels, their clinical target. Nevertheless, several local anesthetic drugs interact with a number of cationic channels including the nicotinic acetylcholine ones. Thus, we used the *Xenopus* oocytes functional expression system to study the effect of benzydamine on a muscle type nicotinic receptor using two electrodes voltage-clamp technique. Acetylcholine (ACh) activated an inward current in oocytes previously (2-10 days) microinjected with cRNA mixture encoding α, β, γ and δ subunits (stoichiometry 2:1:1:1) of the mouse (BC3H1) nicotinic receptor. The amplitude of the ACh-current in presence of benzydamine was reduced in a dose-dependent manner. The benzydamine block was non-competitive with an IC_{50} of 2.4 µM. A further reduction of the maximal current amplitude was achieved perfusing the oocyte for 1 min with benzydamine before ACh (100µM) application. The ACh-current was reduced also by perfusing for 1min with benzydamine followed by 1 min washing in Ringer before the ACh (without benzydamine) application. Moreover, the blockade by benzydamine was largely voltage-independent. At high concentrations of benzydamine (≥10µM) a "use-dependent" block did appear, thus suggesting a block of the close-state of the channel. The present results suggest that benzydamine interacts with the nicotinic acetylcholine receptor at a site related to hydrophobic region of the receptor-channel proteins.

CHARACTERISATION OF PRE- AND POSTSYNAPTIC NICOTINIC ACETYLCHOLINE RECEPTORS.

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On the basis of the results obtained by expression studies, the subunit composition of nicotinic acetylcholine receptors (nAChRs) in different tissue preparations can be deduced by assaying the rank order of potency of different agonists and antagonists. This study attempted to characterise the subunit composition of nAChRs participating in pre- and postsynaptic mechanisms of neural transmission, and to study the role of voltage-sensitive calcium channels in the nAChR-mediated events. For these experiments we studied the effects of a number of nicotinic agonists, including the potent novel analgetic epibatidine, in three different preparations: The guinea pig ileum-myenteric plexus and vas deferens and rat hippocampal slices. We recorded the contraction of the ileal longitudinal muscle to study the postsynaptic effects, and monitored the release of norepinephrine using the superfusion technique to study the presynaptic effects in the latter two preparations. The rank order of potency of agonists suggests that the predominant type of nAChRs present in the presynaptic noradrenergic nerve terminals of the rat vas deferens and hippocampus, as well as those present on other nerve terminals are composed of α3ß2 subunits, whereas in the guinea-pig myenteric plexus the somatodendritic nAChRs are composed of α4ß2 subunits