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Dopamine release in human neocortical slices: Characterization of inhibitory autoreceptors and of nicotinic acetylcholine receptor-evoked release

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Abstract

The autoinhibitory control of electrically evoked release of [^{3}H]-dopamine and the properties of that induced by nicotinic receptor (nAChR) stimulation were studied in slices of the human neocortex. In both models [^{3}H]-dopamine release was action potential-induced and exocytotic. The selective dopamine D_2 receptor agonist (−)-quinpirole reduced electrically evoked release of [^{3}H]-dopamine, yielding IC_{50} and I_{max} values of 23 nM and 76%, respectively. Also, the effects of several other subtype-selective dopamine receptor ligands confirmed that the terminal dopamine autoreceptor belongs to the D_2 subtype. The autoinhibitory feedback control was slightly operative under stimulation conditions of 90 pulses and 3 Hz, with a biophase concentration of endogenous dopamine of 3.6 nM, and was enhanced under blockade of dopamine reuptake. [^{3}H]-dopamine release evoked in an identical manner in mouse neocortical slices was not inhibited by (−)-quinpirole, suggesting the absence of dopamine autoreceptors in this tissue and underlining an important species difference.

Also, nAChR stimulation-induced release of [^{3}H]-dopamine revealed a species difference: [^{3}H]-dopamine release was evoked in human, but not in rat neocortical slices. The nAChRs inducing [^{3}H]-dopamine release most probably belong to the α_3/β_2-subtype, according to the potencies and efficacies of subtype-selective nAChR ligands. Part of these receptors may be located on glutamatergic neurons.

Keywords: Presynaptic dopamine autoreceptors; Presynaptic nicotine receptors; Dopamine release; Human neocortex; Rat neocortex; Mouse neocortex

1. Introduction

Presynaptic metabotropic receptors typically diminish, whereas presynaptic ionotropic receptors mostly enhance the release of neurotransmitters. Activation of ionotropic receptors per se may induce release by depolarisation of nerve terminals due to ion in- or efflux, but activation of metabotropic receptors mostly diminishes exocytosis by influencing – directly or indirectly – depolarisation-gated Ca^{2+} entry. Physiologically, depolarisation results from either propagated action potentials or from the mentioned cation influx through receptor-gated cation channels.

In the central nervous system (CNS), the neurotransmitter dopamine (DA), besides its importance in motor (nigrostriatal DA system) and endocrine functions (tuberoinfundibular DA system), plays also an important role in emotional (mesolimbic DA system) and even cognitive functions (mesocortical DA system). For instance, as regards human pathophysiology, a decline in the function of the prefrontal dopaminergic projection may be related to cognitive deterioration [10]. In support of the latter assumption, the selective DA D_2 receptor agonist quinpirole has been shown to impair prefrontal cortical cognitive function in monkeys due to activation of D_2 autoreceptors [6]. Hence, the presynaptic modulation of cortical DA release may be an important target for the regulation of cognition also in humans.

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In human neocortical tissue, however, modulation of DA release has only rarely been investigated [8,22,59]. Therefore, the first aim of the present study was to characterize the metabotropic autoreceptor on dopaminergic axon terminals in freshly obtained human neocortical tissue. Although this receptor has been previously suggested to belong to the D_2 type of DA receptors [22,23], these authors used a – perhaps misleading – continuous electrical stimulation protocol on human brain slices to induce DA release. Since this technique might exhaust the neurotransmitter pool and thereby also change the responsiveness of the release machinery (see Section 4), we wanted to reinvestigate the type of this autoreceptor using milder conditions of non-continuous electrical field stimulation.

Examples of transmitter release due to opening of receptor-gated cation channels include NMDA receptor-mediated glutamate and ACh release [35,40,45], 5-HT_3 receptor-induced GABA release in the amygdala [37], glycine receptor-evoked ACh release from cholinergic neurons with high intracellular chloride concentration [18,19,21] and nicotinic acetylcholine receptors (nAChR)-evoked release of glutamate or GABA [49] and of a host of other transmitters [63]. Neuronal nAChRs consist of α- and β-subunits, from which nine different α (α_2–α_{10}) and three different β types (β_2–β_4) are known so far; the subunits form a large family of pentameric ligand-gated receptors of various subunit compositions; nAChRs are widely distributed in the CNS where they may differ between regions and species.

In human neocortical tissue, we have recently shown [3] that stimulation of presynaptic nAChRs evokes the release of noradrenaline (NA). We also observed that in the rat neocortex, NA release was not induced by nAChR activation, whereas in the hippocampus of this species the properties of nAChR-induced NA release were similar to those in the human neocortex. Furthermore, nicotinic autoreceptors (i.e. nAChRs on cholinergic terminals) could be excluded both for human and rat neocortical tissue. With respect to subunit composition, $\alpha_3\beta_2$- and/or α_6-containing nAChRs, inducing [^{3}H]-NA release, were found in human neocortex according to the effects of subtype preferring or selective nAChR ligands. Although these nAChRs appeared to be localized mainly presynaptically on noradrenergic axon terminals in the human neocortex, part of the effect of nicotinic agonists was also mediated indirectly, i.e. via stimulation of nAChRs on glutamatergic neurons, followed by ionotropic glutamate receptor-mediated release of NA [3]. Stimulation of nAChRs has also been shown to induce the release of further neurotransmitters [42,63], including that of striatal dopamine of the rat (e.g. [30] or the mouse [62]). Interestingly, the latter author showed that nicotine enhanced striatal DA release by two complementary actions: terminal non-α_7-nAChRs mediated exocytotic DA release and terminal α_7-containing nAChRs mediated a Ca^{2+}-dependent increase in synaptic vesicle availability.

In context with the possible importance of neocortical DA release in the human brain for cognitive functions (see above), it is interesting that nAChRs have been suggested as possible targets for drugs used in Alzheimer's and Parkinson's diseases (e.g. [9,41]). Therefore, it was the second aim of the present investigation to find out whether stimulation of nAChRs affects DA release in the human neocortex and if so, to characterize the localization and subunit composition of the presynaptic nAChRs involved.

Moreover, with respect to the species differences already mentioned above, a third aim of this study was to compare the properties of the presynaptic modulation of DA release in human neocortical tissue with that in the rat or mouse neocortex.

2. Experimental procedures

2.1. Tissue preparation

Fresh human neocortical tissue was obtained from 41 patients (age 5–69 years) during surgical access to subcortical tumours or epilepsy foci. The regions of tissue origin included frontal, temporal, parietal or occipital areas. The procedure was approved by the local Ethical Committee of the University of Freiburg, Germany. The patients were informed about the purpose of the investigation, and signed a declaration of consent.

Mice weighing between 25 g and 35 g each (strain B6 CBA) and Wistar rats (200–300 g) of both sexes were decapitated under anaesthesia with CO_2, brains were quickly removed and neocortical slices were processed exactly as described below for human tissue slices. Animal experiments were conformed to the rules of the German law regulating the use of animals in biomedical research and all efforts were made to minimize both the suffering and the number of animals.

The neocortical tissues were instantly immersed in ice-cold buffer, saturated with 95% O_2, 5% CO_2 which contained (in mM): NaCl 121, KCl 1.8, $CaCl_2$ 1.3, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 11; pH was adjusted to 7.4. The tissue was prepared and cut into 350 μm thick slices using a McIllwain tissue chopper (Bachofer, Reutlingen, Germany). Human tissue macroscopically infiltrated with tumour was excluded. Microscopic tissue infiltration by malignant growth could, moreover, be suspected retrospectively: a significantly lower stimulation-evoked [^{3}H]-efflux was obtained from such slices; consequently, data from these slices were excluded from further evaluation. Drugs given to the patients before and/or during surgery were as described previously [26] and did not appear to modify the evoked [^{3}H]-efflux.

2.2. Tissue accumulation of [^{3}H]-DA

In pilot experiments, mouse, rat and human neocortical slices, 0.35 mm thick and of macroscopically similar size in each species (mouse: 1–3 mm diameter; rat: 2–4 mm; human: 2–4 mm), were incubated in buffer (see above) with (or without) drugs in the additional presence of 0.1 μM [^{3}H]-DA for 15 min at 37 °C. In each experiment 12–16 slices without drugs were compared to 12–16 slices incubated with the noradrenaline uptake blocker (+)-oxaprotiline (1 μM) in addition to the 5-HT uptake inhibitor fluvoxamine (1 μM). During incubation the slices were saturated with 95% O_2/5% CO_2. After incubation the slices were rinsed and dissolved in 0.5 ml Soluene-350 (Packard Instruments, Frankfurt, Germany) for tritium determination.

According to the effects observed in these pilot experiments (see Section 3) slices to be used in all subsequent superfusion experiments were routinely incubated for 45 min in 0.1 μM [^{3}H]-DA in the presence of both (+)-oxaprotiline (1 μM) plus fluvoxamine (1 μM) to avoid false labelling of noradrenergic and serotonergic terminals, respectively.

2.3. Electrically and potassium-evoked release of [^{3}H]-DA

After incubation the slices were rinsed, transferred to small superfusion chambers for electrical stimulation and superfused at 0.4 ml/min. Also, this superfusion medium contained both (+)-oxaprotiline (1 μM) and fluvoxamine (1 μM) to prevent reuptake of released [^{3}H]-DA into noradrenergic and serotonergic terminals during the experiment. After 60 min of continuous washing the superfusate was collected in 5 min samples and the slices were electrically stimulated three times (90 rectangular pulses, 2 ms, 3 Hz, 20–60 mA [as indicated]) after 15 min (S_1), 60 min (S_2) and 100 min (S_3). Tritium overflow was estimated in the absence (controls) and presence of test drugs given, at increasing