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**SPECIAL REPORT**



# Release-regulating dopamine autoreceptors in human cerebral cortex

Ernesto Fedele, *Gian Carlo Andrioli, *Antonio Ruelle & [1]Maurizio Raiteri

Institute of Pharmacology and Pharmacognosy, University of Genova, Viale Cembrano 4, 16148 Genova and *Division of Neurosurgery, Galliera Hospital, Via A. Volta 8, 16128 Genova, Italy

Slices from fresh specimens of human neocortex which had to be removed during neurosurgery to reach subcortical tumours were labelled with [$^3$H]-dopamine and stimulated electrically. Quinpirole, a selective dopamine $D_2$ receptor agonist, inhibited the stimulated tritium overflow ($EC_{50}$ = 25 nM; maximal inhibition: about 80% at 10 μM). The selective $D_1$ receptor agonist, SKF 38393, was inactive up to 10 μM. Quinpirole was antagonized by the $D_2$ receptor antagonist (−)-sulpiride (apparent $pA_2$ = 8.26). Thus dopaminergic axon terminals in the human mesocortical pathway possess autoreceptors of the $D_2$ type.

**Keywords:** Human neocortex; dopamine release; dopamine autoreceptors; dopamine $D_2$ receptors

**Introduction** Receptor heterogeneity bears important implications. The phenomenon favours the development of more selective drugs. However, the increasing evidence that different isoreceptors subserve identical function in different species requires the development of novel drugs based on animal studies to be postponed until the human target receptors are characterized.

Autoreceptors on presynaptic nerve terminals participate in the local control of transmitter release. According to a review by Starke *et al.* (1989), dopamine release-modulating autoreceptors exist in various brain areas of different animal species and belong to the $D_2$ type. Most studies have been performed in the mesostriatal system, whereas few investigations concern dopamine autoreceptors in the terminal regions of the mesocortical dopaminergic pathway.

With the exception of one study on dopamine autoreceptors in *post-mortem* human striatum (Hetey *et al.*, 1991), no data are available regarding the presence of these release-regulating receptors in human brain. Actually, according to De Keyser (1993), the available data do not support the existence of such presynaptic receptors in man. We have therefore investigated the presence and the pharmacological characteristics of dopamine receptors able to regulate [$^3$H]-dopamine release from electrically-stimulated slices of human neocortex.

**Methods** Specimens of human cerebral cortex were obtained from patients undergoing neurosurgery to remove subcortical tumours. Samples of frontal (1), temporal (4) and parietal (2) cortex from 5 males and 2 females (aged 32–57 years) were used. After removal, the tissue was kept in ice-cold medium containing (mM): NaCl 125, KCl 3, $MgSO_4$ 1.2, $CaCl_2$ 1.2, $NaHCO_3$ 22, $Na_2HPO_4$ 1 and glucose 10, aerated with $O_2$/$CO_2$ (95:5), pH 7.4

Slices (approx. 4 × 4 × 0.4 mm) were incubated (15 min, 37°C) with 0.02 μM [$^3$H]-dopamine in the presence of 0.1 μM 6-nitroquipazine and nisoxetine to prevent false labelling of 5-hydroxytryptaminergic and noradrenergic terminals, respectively. Slices were then transferred into 12 parallel superfusion chambers (1 slice/chamber) and stimulated according to a continuous electrical stimulation protocol (Raiteri *et al.*, 1992).

Each experiment was carried out on tissue obtained from a single patient; full concentration-response curves for the agonist(s), in the presence or in the absence of (−)-sulpiride, along with appropriate controls were done in the same experiment.

[1] Author for correspondence.

The tritium present in each 5 min fraction (see Figure 1) was calculated as a percentage of the total tissue content at the onset of the fraction collected. The evoked $^3$H overflow in the fraction $F_1$ and in each fourth fraction collected after addition of the various agonist concentrations ($F_2$, $F_3$, $F_4$, $F_5$) was calculated by subtracting basal efflux from total efflux in the fraction considered.

The agonist effects on the evoked $^3$H overflow were evaluated by comparing the ratios $F_2/F_1$, $F_3/F_1$, $F_4/F_1$, $F_5/F_1$ to the corresponding ratios obtained under control conditions, i.e.

$$\% \text{ change} = 100 \times \left( \frac{F_x/F_1 \text{ agonist}}{\text{average } F_x/F_1 \text{ control}} - 1 \right)$$

No significant differences both in the evoked overflows and in the effects of drugs have been observed among the different cortical areas. Therefore the data obtained from the different experiments were pooled.

The $EC_{50}$ values for quinpirole were determined graphically at the 40% level of inhibition, the maximum effect of the

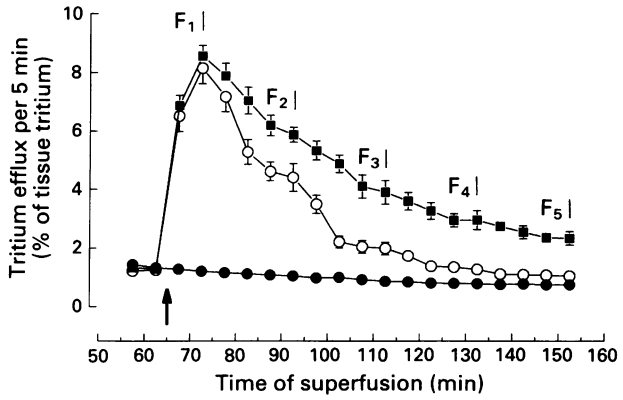


**Figure 1** Continuous electrically-evoked release of tritium from human cerebral cortex slices labelled with [$^3$H]-dopamine. Slices were labelled and superfused at 1 ml min$^{-1}$. Electrical stimulation (3 Hz, 2 ms, 24 mA) was applied from $t$ = 65 (see arrow) to the end of the experiment. Increasing concentrations of agonists (0.01, 0.1, 1 and 10 μM) were added every 20 min ($t$ = 75, 95, 115, 135 min). $F_1$, $F_2$, $F_3$, $F_4$ and $F_5$ represent the fractions at which the $^3$H overflow was calculated. Each point represents the mean ± s.e.mean of 7 experiments in duplicate: (●) spontaneous $^3$H outflow; (■) evoked $^3$H efflux; (○) evoked efflux in the presence of quinpirole.

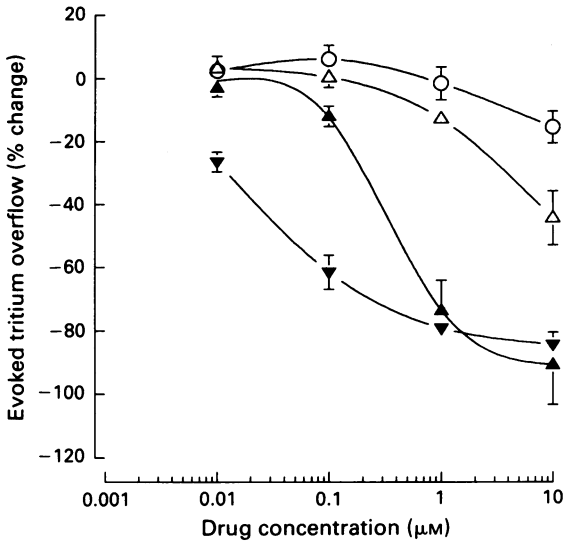


**Figure 2** Effects of quinpirole and SKF 38393 on the electrically-evoked tritium overflow from human neocortex slices and antagonism by (−)-sulpiride. Drugs were added every 20 min after the peak of the evoked tritium efflux had been reached. (−)-Sulpiride was present throughout the experiment. For further details see Methods. Means ± s.e.mean of 3–7 experiments in duplicate are shown: (▼) quinpirole; (○) SKF 38393; (▲) quinpirole + 0.1 μM (−)-sulpiride; (△) quinpirole + 1 μM (−)-sulpiride.

agonist being about 80%. The apparent $pA_2$ values of (−)-sulpiride, calculated according to Furchgott (1972, p. 290) for the two concentrations of the antagonist, were averaged.

Student's *t* test was used to analyse the significance of the difference between two means.

*Drugs* [$^3$H]-dopamine (specific activity 45 Ci mmol$^{-1}$) was purchased from Amersham Radiochemical Centre (Buckinghamshire). The following drugs were gifts from the companies indicated: 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-benzazepine (SKF 38393) (SmithKline Beecham, Surrey); quinpirole (Eli Lilly & Co, Indianapolis, IN, U.S.A.); (−)-sulpiride (Ravizza, Milan, Italy).

**Results** Figure 1 illustrates the patterns of tritium release from human cortical slices prelabelled with [$^3$H]-dopamine. The spontaneous outflow in the pre-stimulation fraction was 1.294 ± 0.112%. The tritium overflows at $F_1$, $F_2$, $F_3$, $F_4$ and $F_5$, under control conditions, amounted to 7.415 ± 0.460, 4.897 ± 0.641, 3.065 ± 0.530, 2.194 ± 0.418 and 1.667 ± 0.299%, respectively. The figure also shows the effects of quinpirole added at increasing concentrations in order to construct concentration-response curves (see Figure 2).

Quinpirole (0.01–10 μM) inhibited the overflow of tritium from slices prelabelled with [$^3$H]-dopamine. The maximal inhibition (about 80%) was reached at 10 μM. The $EC_{50}$ value amounted to 25 nM. No significant changes of tritium overflow were produced by SKF 38393. Addition of (−)-sulpiride (0.1 and 1 μM) shifted to the right the concentration-response curve of quinpirole. The $pA_2$ average value was 8.26 (8.51 at 1 μM and 8.02 at 0.1 μM).

Under our experimental conditions, (−)-sulpiride, at 1 μM, did not affect on its own the tritium overflow ($F_1$ = 7.906 ± 1.487; $F_2$ = 4.864 ± 1.299; $F_3$ = 3.127 ± 0.184; $F_4$ = 2.049 ± 0.565; $F_5$ = 1.482 ± 0.462%; $n$ = 4). The drugs used had no significant effect, on their own, on basal tritium outflow (not shown).

**Discussion** Mesocortical dopaminergic neurones seem to play an important role in the therapeutic activity of antipsychotics which justifies investigations into the mechanisms regulating the activity of dopaminergic neurones projecting into the cortex. Studies of autoreceptors regulating dopamine release in animal neocortex are surprisingly few (Plantjé *et al.*, 1985; Talmaciu *et al.*, 1986; Hoffman *et al.*, 1988) as compared to those on dopamine autoreceptors in the mesostriatal pathway (see review by Starke *et al.*, 1989). Furthermore, the existence of dopamine autoreceptors in human cortex has not been reported.

Quinpirole, a selective agonist at dopamine $D_2$ receptors, but not the $D_1$ agonist SKF 38393, inhibited the overflow of [$^3$H]-dopamine from human neocortex slices. Accordingly, the selective $D_2$ receptor antagonist (−)-sulpiride prevented the inhibition by quinpirole. The results indicate the existence of dopamine autoreceptors which may be pharmacologically classified as the $D_2$ type.

An apparent $pA_2$ value of 8.26 was obtained for (−)-sulpiride as a dopamine autoreceptor antagonist in human cortex. Curiously enough, in spite of the wide use of this drug as a diagnostic $D_2$ receptor antagonist, to our knowledge the only quantitative evaluation for (−)-sulpiride as a blocker of dopamine autoreceptors was carried out in the rabbit caudate nucleus ($pA_2$ = 7.84; Starke *et al.*, 1983).

(−)-Sulpiride did not increase, on its own, the evoked overflow of [$^3$H]-dopamine, a result often interpreted as absence of tonic activation. Although a definite conclusion requires further experiments, it is worth noting that (−)-sulpiride behaved similarly in slices of rabbit frontal cortex, whereas it increased [$^3$H]-dopamine overflow in the striatum (Hoffman *et al.*, 1988).

To conclude, the release of dopamine from mesocortical axon terminals in human brain is sensitive to $D_2$ receptor ligands suggesting the presence of presynaptic autoreceptors of the $D_2$ type. According to molecular biology studies, subtypes of the $D_2$ receptor exist in man (De Keyser, 1993); however, a pharmacological subclassification of the dopamine autoreceptor in man will not be possible until novel selective drugs are available.

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