arrested crack fully extends through the compressive layer in a straight path, whereas experimentally the crack is observed to bifurcate. Fracture in subsequent layers is reinitiated by a new crack and not by the original crack. Therefore, despite the fair agreement of the predicted and measured threshold strengths, it is still not certain that Eq. 3 accurately estimates the value of the threshold strength. Further experiments will be needed to fully understand this important phenomenon. In addition, the effect of the elastic moduli of the two materials, not considered here, is under consideration. However, it is expected that the phenomenon described here can be applied to other brittle materials, including glasses, many polymers, and some metals.

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Enhanced Cortical Dopamine Output and Antipsychotic-like Effects of Raclopride by α_2 Adrenoceptor Blockade

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Clozapine exerts superior clinical efficacy and markedly enhances cortical dopamine output compared with classical antipsychotic drugs. Here the α_2 adrenoceptor antagonist idazoxan was administered to rats alone or in combination with the $D_{2/3}$ dopamine receptor antagonist raclopride. Dopamine efflux in the medial prefrontal cortex and conditioned avoidance responding were analyzed. Idazoxan selectively potentiated the cortical output of dopamine and augmented the suppression of conditioned avoidance responding induced by raclopride. These results challenge basic assumptions underlying the dopamine hypothesis of schizophrenia and provide insight into clozapine's mode of action.

The dopamine hypothesis of schizophrenia rests basically upon the fact that all hitherto discovered antipsychotic drugs have been found to antagonize dopamine neurotransmission in brain (*1*). Positron emission tomography studies in humans reveal that classical antipsychotics in clinically effective doses yield about 70 to 80% D_2 receptor occupancy in striatal tissue, which approaches the threshold for extrapyramidal side effects (*2*). In preclinical studies all antipsychotic drugs suppress conditioned avoidance responding (*3*, *4*), a behavioral paradigm that has been attributed to drug effects on the subcortical, mesolimbic dopamine projection (*5*). The dopamine hypothesis of schizophrenia is, however, profoundly challenged by the atypical antipsychotic drug clozapine, which shows significantly better efficacy than classical antipsychotics including an improved effect on negative symptoms (*6*), despite a lower D_2 receptor occupancy in brain [that is, ~45 to 50% (*2*)]. Clozapine accordingly has fewer extrapyramidal side effects (*7*). In contrast to classical antipsychotic drugs, clozapine causes a marked increase in dopamine output in the medial prefrontal cortex (*8*), an effect of considerable interest because of the role of prefrontal dopamine in cognitive functioning (*9*). Indeed, recent clinical evidence indicates that the degree of cognitive impairment largely determines treatment outcome in schizophrenia, in particular with regard to social functioning (*10*).

Clozapine has considerable affinity for α_2 adrenoceptors (*11*), and clinical trials demonstrate that adjuvant treatment with α_2 adrenoceptor antagonists may augment the clinical efficacy of classical D_2 receptor–blocking drugs (*12*). Accordingly, the α_2 adrenoceptor blocking effect of antipsychotic drugs, such as clozapine and risperidone, has been hypothesized to be important for their clinical profiles (*13*).

We thus investigated the effects of combining a low dose of idazoxan, a specific α_2 adrenoceptor antagonist (*14*), with raclopride, a selective $D_{2/3}$ receptor antagonist (*15*), on dopamine output in the prefrontal cortex compared with subcortical regions of the brain. We also tested the effect of the drug combination on conditioned avoidance responding, a preclinical test with high predictive validity for a clinical antipsychotic effect (*3*, *4*). We then tested the effect of the drug combination on catalepsy scores, a preclinical assessment of extrapyramidal side-effect liability (*16*).

Raclopride alone [0.05 mg per kilogram of body weight, subcutaneous injection (sc)] induced a marked increase in dopamine output as measured by microdialysis (*17*) in the nucleus accumbens and striatum, but caused only a small increase in the dopamine efflux in the medial prefrontal cortex (Fig. 1) (*8*). Idazoxan (1.5 mg/kg, sc) alone enhanced dopamine output in the medial prefrontal cortex and largely potentiated the raclopride-induced effects in the medial prefrontal cortex, whereas the subcortical areas remained unaffected (Fig. 1). This failure of idazoxan to augment the raclopride-induced increase in the subcortical areas is unlikely to be a ceiling effect, because a higher dose of raclopride (2.0 mg/kg, sc) further increased dopamine output in these brain regions (*18*). The regional selectivity of idazoxan on dopamine efflux is consistent with data from other, nonselective α_2 adrenoceptor antagonists (*19*) and may be indirectly related to its ability to facilitate cortical noradrenaline efflux (*20*). The noradrenaline transporter contributes to the clearance of dopamine from the extracellular compartment within the cortex (*21*). The concentrations of extracellular noradrenaline may affect cortical dopamine levels by competing for the same transporter.

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Therefore, the augmenting effect on cortical dopamine output induced by cotreatment with idazoxan and raclopride may be the result of a D_2 receptor–mediated increase in dopamine release, combined with an α_2 adrenoceptor–mediated increase in noradrenaline efflux, with a subsequent reduction of dopamine removal from the extracellular space in the medial prefrontal cortex.

Administration of raclopride alone suppressed the conditioned avoidance response in a dose-dependent manner (Fig. 2) (*22*, *23*) and caused pronounced cataleptic behavior (Fig. 3) (*24*). Although ineffective by itself, pretreatment with idazoxan (1.5 mg/kg, sc) significantly augmented the suppression of conditioned avoidance response induced by even low doses of raclopride (0.05 and 0.01 mg/kg, sc). However, pretreatment with idazoxan did not affect the raclopride-induced catalepsy (Fig. 3). Both of the drug-induced effects on conditioned avoidance response and on catalepsy returned to baseline within 240 min after raclopride administration. The lack of effect on catalepsy indicates that the idazoxan-induced augmentation of conditioned avoidance response produced by raclopride is not caused by a general impairment of behavioral performance or a pharmacokinetic interaction between these compounds.

The clozapine challenge to the classical dopamine hypothesis of schizophrenia may not necessarily involve facilitation of dopaminergic functions in the prefrontal cortex, because clozapine also exerts an antagonistic effect at other than D_2 dopamine receptors, for example, D_4 receptors in cortical areas (*25*). However, our results suggest a potent, classical antipsychotic effect of the combined D_2/α_2 receptor antagonist that is indeed associated with an augmented dopaminergic neurotransmission in the prefrontal cortex, because idazoxan does not block any dopamine receptors in brain (*14*). This classical antipsychotic-like effect of the combined D_2/α_2 receptor blockade can be achieved at a substantially lower dose of raclopride (0.05 mg/kg) than would otherwise be possible, which in the rat may yield an estimated D_2 receptor occupancy of less than 5% (*26*). A two- to fourfold higher dose of raclopride alone was required to cause a similar suppression of the conditioned avoidance response. Our results thus indicate that the suppression of conditioned avoidance responding by clozapine, which can be effectively achieved with doses less than 5 mg/kg (*27*), may be related to its α_2 adrenoceptor blocking action. Clozapine, even within this dose range, yields an estimated D_2 receptor occupancy in the rat brain of about 25% (*28*). The D_2/α_2 receptor antagonist combination may permit an even more effective antipsychotic-like effect than clozapine, but obtained at a lower D_2 receptor occupancy. Importantly, our results also indicate that the selectively enhanced dopamine output in the prefrontal cortex may be causally related to this phenomenon.

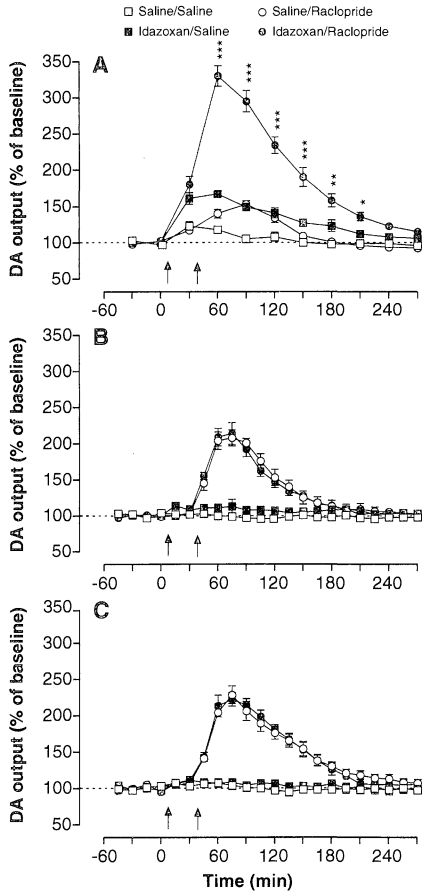

Fig. 1. Effects of saline or raclopride (0.05 mg/kg, sc) administration on dopamine output in (**A**) the medial prefrontal cortex, (**B**) nucleus accumbens, and (**C**) striatum in rats pretreated (30 min) with saline or idazoxan (1.5 mg/kg, sc). Arrows indicate time of injections. Each point represents the mean ± SEM percent change from baseline ($n = 5$ in all groups). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ for comparisons between Saline/Raclopride and Idazoxan/Raclopride treatment groups. Two-way analysis of variance (ANOVA; treatment × time) with repeated measures followed by Newman-Keuls test for multiple comparisons was used to determine significance.

Our data challenge the assumption that suppression of conditioned avoidance responding may help to select only drugs with a classical antipsychotic profile (*4*), such as D_2 receptor antagonists with a relatively high propensity to produce extrapyramidal side effects. Because clinical results show augmentation by idazoxan of classical antipsychotics (*12*), our data suggest that the conditioned avoidance response paradigm, in contrast to previous assumptions, may prove instrumental in the discovery of novel, atypical antipsychotic compounds.

The mechanism by which α_2 adrenoceptor blockade augments the antipsychotic effect of D_2 receptor antagonists may involve other

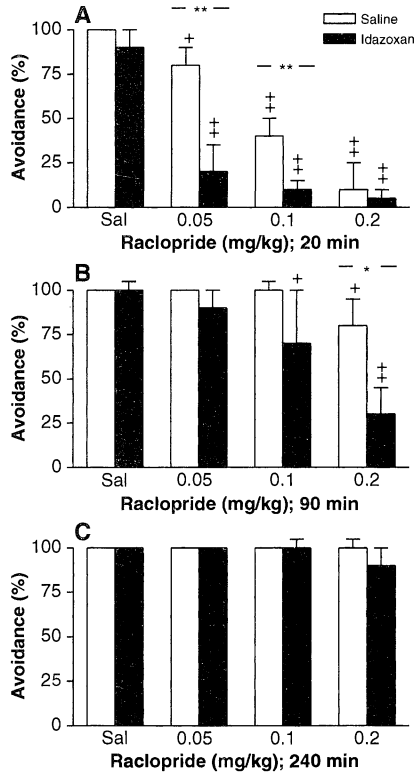

Fig. 2. Effects of saline or idazoxan (1.5 mg/kg, sc) pretreatment (10 min) on saline- or raclopride-induced (0.05, 0.1, and 0.2 mg/kg, sc) effects in the conditioned avoidance response assay (**A**) 20 min, (**B**) 90 min, and (**C**) 240 min after saline or raclopride administration. Bars represent the median percent (± semi-interquartile range) avoidance ($n = 11$ in all groups). $^{*}P < 0.05$, $^{**}P < 0.01$ for comparisons between Saline/Raclopride and Idazoxan/Raclopride treatment groups and $^{+}P < 0.05$, $^{++}P < 0.01$ compared with the respective control group, that is, the Saline/Saline or Idazoxan/Saline treatment group. Friedman's ANOVA followed by Wilcoxon's signed ranks test was used to determine significance.

neurotransmitters. Idazoxan also enhances the release of noradrenaline (*20*) and serotonin (*29*). However, blockade of brain noradrenergic neurotransmission by α_1 adrenoceptor antagonists enhances the effect of D_2 receptor antagonists on conditioned avoidance responding (*4*, *30*). The effects of a generally augmented serotonergic neurotransmission for antipsychotic efficacy remain controversial (*31*).

Schizophrenia may indeed be associated with an impaired dopaminergic functioning in the prefrontal cortex (*32*). Reduced dopamine utilization in the prefrontal cortex has been described in monkeys subjected to chronic treatment with the schizophrenomimetic noncompetitive *N*-methyl-D-aspartate receptor antagonist phencyclidine (*33*). Thus, enhanced dopamine output in the prefrontal cortex, such as that induced by α_2-adrenoceptor blockade, may have therapeutic value for the treatment of

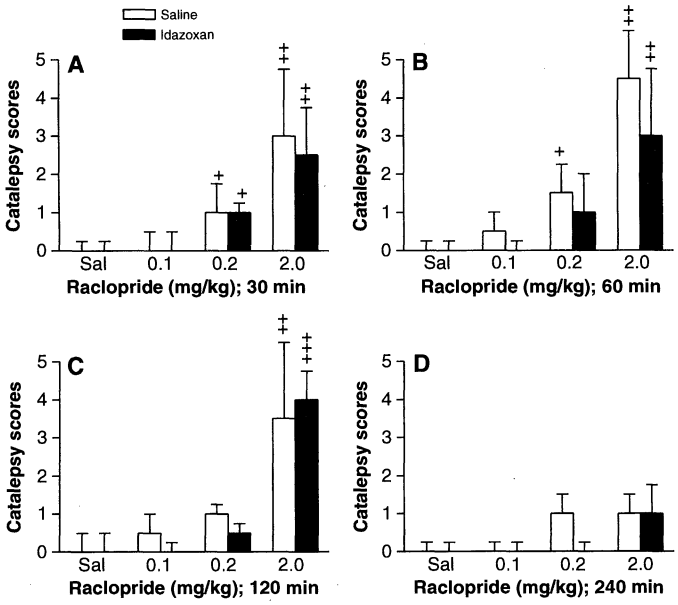

Fig. 3. Effects of saline or idazoxan (1.5 mg/kg, sc) pretreatment (10 min) on saline- or raclopride-induced (0.1, 0.2, and 2.0 mg/kg, sc) catalepsy (**A**) 30 min, (**B**) 60 min, (**C**) 120 min, and (**D**) 240 min after saline or raclopride administration. Bars represent the median (± semi-interquartile range) catalepsy score ($n = 8$ in all groups). $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ compared with the respective control group, that is, the Saline/Saline or Idazoxan/Saline treatment group. Kruskal-Wallis ANOVA followed by Mann-Whitney U-test was used to determine significance.

deficits in cognitive functioning and goal-oriented behavior. Indeed, clinical results suggest that idazoxan treatment improves cognitive performance in dementia of the frontal type (*34*). One underlying mechanism may involve activation of the D_1 receptor, because D_1 receptor agonists alleviate cognitive deficits induced by lesions of the dopaminergic system (*35*). Moreover, recent behavioral experiments suggest that clozapine may also exert some D_1 receptor agonistic effect (*36*).

Our results provide support for ongoing reassessment of the dopamine hypothesis of schizophrenia and may help to further understand the pharmacological basis of the atypical antipsychotic profile of clozapine. Our data also challenge concurrent views on the predictive and mechanistic aspects of the conditioned avoidance response paradigm, which have been accepted since the introduction of neuroleptics. Finally, our study proposes that the α_2 adrenoceptor antagonistic action of clozapine may have a significant bearing on its superior antipsychotic efficacy.

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23. The experiment was performed as described (*5*). In a conventional shuttle-box divided into two compartments, rats were trained to move into the adjacent compartment (response) within 10 s upon presentation of the conditioned stimulus (80 dB white noise) in order to avoid the unconditioned stimulus, an intermittent electric shock in the grid floor. Avoidance was defined as response to the conditioned stimulus within 10 s, and escape as no response to the conditioned stimulus within 10 s but response to the unconditioned stimulus within 60 s. Each training session consisted of 20 trials randomly distributed over 15 min. Only rats that performed avoidance in >80% of the trials after the training sessions were included in subsequent experiments. Experimental sessions were preceded by a pretest session to reaffirm maintenance of the avoidance response. All pretests and experimental sessions consisted of 10 trials randomly distributed over 7.5 min. The avoidance response was calculated and presented as the median (± semi-interquartile range) percent avoidance (the percentage of trials in each session in which the animal performed avoidance). No failures were recorded under any treatment conditions; hence a decrease in avoidance performance was always accompanied by a corresponding increase in escapes.
24. The experiment was performed as described [S. Ahlenius and V. Hillegaart, *Pharmacol. Biochem. Behav.* **24**, 1409 (1986)]. Animals were placed on an inclined (60°) grid and, excluding the first 30 s, the time during which the rat remained in the same position was measured for a maximum of 2.5 min. The catalepsy was scored from 0 to 5 according to the immobility time (square root transformation); score 0 = 0 − 0.08 min, 1 = 0.09 − 0.35 min, 2 = 0.36 − 0.80 min, 3 = 0.81 − 1.42 min, 4 = 1.43 − 2.24 min, and 5 ≥ 2.25 min. The catalepsy scores were presented as medians (± semi-interquartile range).
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