Piribedil Enhances Frontocortical and Hippocampal Release of Acetylcholine in Freely Moving Rats by Blockade of \( \alpha_{2A} \)-Adrenoceptors: A Dialysis Comparison to Talipexole and Quinelorane in the Absence of Acetylcholinesterase Inhibitors

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ABSTRACT
In a dialysis procedure not requiring perfusate addition of acetylcholinesterase inhibitors to “boost” basal levels of acetylcholine (ACh), the influence of the antiparkinson agent piribedil upon levels of ACh in frontal cortex and dorsal hippocampus of freely moving rats was compared with those of other antiparkinson drugs and selective ligands at \( \alpha_{2A} \)-adrenoceptors (ARs). Suggesting a tonic, inhibitory influence of \( \alpha_{2A} \)-ARs upon cholinergic transmission, the \( \alpha_{2A} \)-AR agonist 5-bromo-6-[2-imidazolin-2-yl-amino]-quinoxaline tartrate (UK14,304), and the preferential \( \alpha_{2A} \)-AR agonist guanabenz reduced levels of ACh. They were elevated by the antagonists 2(2-methoxy-1,4-benzodioxan-2-yl)-2-imidazoline HCl (RX821002) and atipamezole and by the preferential \( \alpha_{2A} \)-AR antagonist 2-(2H-(1-methyl-1,3-dihydroisoindole)methyl)-4,5-dihydropyrimazole (BRL44008). In contrast, \( \alpha_{2B/2C} \)-AR antagonists, were inactive. The dopaminergic agonist and antiparkinson agent piribedil, which behaves as an antagonist at \( \alpha_{2} \)-ARs, dose dependently increased extracellular levels of ACh. This action was absent upon pretreatment with a maximally effective dose of RX821002. On the other hand, a further dopaminergic agonist and antiparkinson agent, talipexole, which possesses agonist properties at \( \alpha_{2} \)-ARs, dose dependently reduced levels of ACh. This action was also blocked by RX821002. In contrast to piribedil and talipexole, quinelorane, which interacts with dopaminergic receptors but not \( \alpha_{2} \)-ARs, failed to affect ACh levels. Finally, in analogy to the frontal cortex, piribedil likewise elicited a dose-dependent increase in extracellular levels of ACh in the dorsal hippocampus. In conclusion, in distinction to talipexole and quinelorane, and reflecting its antagonist properties at \( \alpha_{2A} \)-ARs, piribedil reinforces cholinergic transmission in the frontal cortex and dorsal hippocampus of freely moving rats. These actions may be related to its facilitatory influence upon cognitive function.

In Parkinson’s disease (PD), progressive degeneration of nigrostriatal dopaminergic pathways results in a profound disruption of motor function, including such cardinal features as rigidity, bradykinesia, and an inability to initiate movement (Jenner, 1995). In addition, patients frequently reveal sensory deficits, depressed mood, and a perturbation of cognitive function. Although the dopamine (DA) precursor L-dihydroxyphenylalanine (L-DOPA) is universally used in the treatment of PD, certain motor symptoms, as well as the accompanying mnemonic, sensory, and emotional deficits, are little improved (Jenner, 1995). Furthermore, L-DOPA may elicit pronounced dyskinesias (Jenner, 1995). Most disturbingly, its actions eventually become variable with abrupt transitions between “on” (effective) and “off” (ineffective) phases. These observations underpin interest in dopaminergic agents for the management of PD. Although they elicit their own spectrum of side effects (hallucinations, sleep-attacks, and sedation), their low dyskinetic potential and potential neuroprotective properties render them attractive as alternatives (or adjuncts) to L-DOPA, in particular in younger patients (Jenner, 1995; Rascol et al., 2000). The improvement of motor function may primarily be attributed to activation of postsynaptic D\(_2\) receptors in the basal ganglia (Jenner, 1995; Wang et al., 2000). Although D\(_4\) receptors are not of major significance, it remains unclear whether engagement of their D\(_3\) counterparts is advantageous or deleterious in the management of PD (Newman-Tancredi et al., 2002a).

In fact, antiparkinson agents do not exclusively interact

ABBREVIATIONS: PD, Parkinson’s disease; DA, dopamine; L-DOPA, L-dihydroxyphenylalanine; AR, adrenoceptor; FCX, frontal cortex; ACHE, acetylcholinesterase; ACh, acetylcholine; UK14,304, 5-bromo-6-[2-imidazolin-2-yl-amino]-quinoxaline tartrate; BRL41992 maleate, trans-2,3,9,13b-tetrahydro-1,2-dimethyl-1H-dibenzo[c,f]imidazo[1,5-a]azepine; BRL44008 base, 2-(2H-(1-methyl-1,3-dihydroisoindole)methyl)-4,5-dihydropyrimazole; RX821002, 2-(2-methoxy-1,4-benzodioxan-2-yl)-2-imidazoline HCl.
ergic agonists and L-DOPA in rodent and primate models of PD (Sandyk and Iacono, 1990) and, in experimental models, degeneration of adrenergic pathways aggravates PD (Bezard et al., 1998; Bezard et al., 2001). These actions may contribute to cognitive deficits and the perturbation of mood (Rosin et al., 1996; Buclin et al., 1998; Bezard et al., 2001). They also reinforce corticolimbic release of ACh in rats. The objectives of this study were, thus, as follows. First, by use of a procedure not requiring the use of AChE inhibitors (Ichikawa et al., 2000, 2002), we characterized the influence of agonists and antagonists possessing contrasting affinities at α2-AR subtypes (Table 1) upon extracellular levels of ACh in the FCX of conscious rats. Second, the influence of piribedil upon ACh levels in FCX was compared with the effects of talipexole and of quinelorane, the latter a potent dopaminergic agonist lacking affinity at α2-ARs (Table 1; Millan et al., 2002; Newman-Tancredi et al., 2002a,b). In a parallel experiment, their influence upon extracellular levels of DA in this structure was also examined. Finally, the influence of piribedil, compared with α2-AR ligands, upon levels of ACh in the dorsal hippocampus was evaluated.

### Materials and Methods

**Animals.** Male Wistar rats (Iffa Credo, l’Arbresle, France) of 225 to 250 g were allowed free access to food and water and housed singly. Laboratory temperature was 21 ± 1°C and humidity 60 ± 5%. There was a 12-h light/dark cycle (lights on at 7:30 AM). All animal use procedures conformed to international European ethical standards (86/609-EEC) and the French National Committee (décret 87/848) for the care and use of laboratory animals.

**Dialysis Procedure.** Surgery was performed under pentobarbital anesthesia (60 mg/kg i.p.). As described previously (Millan et al., 2001), rats were mounted in a Kopf stereotaxic frame and a single guide cannulae (CMA/11) implanted in the FCX or dorsal hippocampus with coordinates as follow: AP, +2.2; L, 0.6; DV, −0.2; or AP, −3.8; L, 2.0; DV, −2.0, respectively. Rats were single-housed and allowed to recover for 5 days before dialysis. On the day of dialysis, each rat received a 10 min intraperitoneal injection of saline alone or saline containing 2 mg/kg atropine sulfate. After a 5 min pre-measurement phase, a 10 mm length dialysis probe was lowered into place. AChE inhibitors (200 μg) were applied to the dialysis probe for 30 min. AChE activity was determined and, with few exceptions (Cuadra and Giacobini, 1995; DeBoer and Abercrombie, 1996; Ichikawa et al., 2000, 2002), dialysis studies have resorted to AChE inhibitors to “boost” otherwise undetectable basal levels of ACh (Toide and Arima, 1989; Liu and Kato, 1994; Sarter and Bruno, 1998; Shirazi-Southall et al., 2002).

In light of the above-mentioned observations, we hypothesized that, in analogy to α2-AR antagonists, piribedil should reinforce corticolimbic release of ACh in rats. The objectives of this study were, thus, as follows. First, by use of a procedure not requiring the use of AChE inhibitors (Ichikawa et al., 2000, 2002), we characterized the influence of agonists and antagonists possessing contrasting affinities at α2-AR subtypes (Table 1) upon extracellular levels of ACh in the FCX of conscious rats. Second, the influence of piribedil upon ACh levels in FCX was compared with the effects of talipexole and of quinelorane, the latter a potent dopaminergic agonist lacking affinity at α2-ARs (Table 1; Millan et al., 2002; Newman-Tancredi et al., 2002a,b). In a parallel experiment, their influence upon extracellular levels of DA in this structure was also examined. Finally, the influence of piribedil, compared with α2-AR ligands, upon levels of ACh in the dorsal hippocampus was evaluated.

### Summary of Drug Pharmacological Profiles

<table>
<thead>
<tr>
<th>Drug</th>
<th>Activity</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>UK14,304</td>
<td>α2-AR agonist</td>
<td>Renouard et al. (1994)</td>
</tr>
<tr>
<td>Atipamezole</td>
<td>α2-AR antagonist</td>
<td>Renouard et al. (1994)</td>
</tr>
<tr>
<td>RX821002</td>
<td>α2-AR antagonist</td>
<td>Renouard et al. (1994)</td>
</tr>
<tr>
<td>Guanabenz</td>
<td>Preferential α2A-AR agonist</td>
<td>Renouard et al. (1994)</td>
</tr>
<tr>
<td>BRL14468</td>
<td>Preferential α2A-AR agonist</td>
<td>Young et al. (1989)</td>
</tr>
<tr>
<td>BRL14992</td>
<td>Preferential α2C-AR antagonist</td>
<td>Young et al. (1989)</td>
</tr>
<tr>
<td>Prazosin</td>
<td>Preferential α2C-AR (and α1-AR) antagonist</td>
<td>Renouard et al. (1994)</td>
</tr>
<tr>
<td>Pirebidil</td>
<td>α2-AR antagonist and D2 agonist</td>
<td>Millan et al. (2002)</td>
</tr>
<tr>
<td>Talipexole</td>
<td>α2-AR agonist and D2 agonist</td>
<td>Millan et al. (2002)</td>
</tr>
<tr>
<td>Quinelorane</td>
<td>D2 agonist</td>
<td>Millan et al. (2002)</td>
</tr>
</tbody>
</table>
a cuprophan CMA/11 probe (4 mm in length for the FCX and 2 mm in length for the dorsal hippocampus, 0.24 mm o.d.) was slowly lowered into position. It was perfused at 1 µl/min with a phosphate-buffered solution of 147.2 mM NaCl, 4 mM KCl, and 2.3 mM CaCl₂, pH 7.3. Two hours after implantation, 20-min dialysate samples were collected for 3 h. Three basal samples were collected before drug administration. In the antagonist studies, RX821002 was injected 20 min before piribedil or talipexole. The influence of drugs and vehicle was expressed relative to basal values (defined as 0%).

**Chromatographic Procedures.** ACh was quantified in the absence of AChE inhibitors, essentially as described by Ichikawa et al. (2000). Twenty-microliter dialysate samples were collected on 10 µl of 0.01% acetic acid. Twenty-microliter aliquots were then analyzed by high-performance liquid chromatography. The mobile phase was composed of 50 mM Na₂HPO₄ and 0.5% prolin (BAS, Congleton, UK), adjusted to pH 8.2 with H₃PO₄. The stationary phase was comprised of a cation ion exchanger (Sepstik, 530 × 1.0 mm, particle size 10 µm; BAS), a precolumn (preimmobilized enzyme reactor, 55 × 1 mm) of choline oxidase/catalase (BAS), and a postcolumn (postimmobilized enzyme reactor, 50 × 1 mm) of choline oxidase/AChE (BAS) maintained at 35°C. An amperometric detector (LC-4B; BAS) was used for quantification. The electrode was set at +100 mV versus Ag/AgCl. The glassy carbon electrode (MF2098, BAS) was coated with the peroxidase-redox polymer. The mobile phase was delivered at a flow rate of 0.14 ml/min. The sensitivity of the assay for ACh was 0.1 pg (0.55 fmol) (injected in a volume of 20 µl). DA levels were quantified by high-performance liquid chromatography followed by coulometric detection as described previously (Millan et al., 2001). The assay limit of sensitivity was 0.1 pg/sample. Data were analyzed by ANOVA with sampling time as the repeated within-subject factor.

**Chemicals and Drugs.** All drugs were injected s.c. in a volume of 1.0 ml/kg. Drugs were dissolved in sterile water plus a few drops of lactic acid if necessary and the pH adjusted to >5.0. Guanabenz base, quinolone 2HCl, 2(2-methoxy-1,4 benzodioxan-2-yl)-2-imidaizolone HCl (RX821002), prazosin HCl and talipexole 2HCl were purchased from Sigma Chemie (Chesnes, France). Atipamezole HCl, zolines HCl (RX821002), prazosin HCl and talipexole 2HCl were base, quinelone 2HCl, 2(2-methoxy-1,4 benzodioxan-2-yl)-2-imidaizolone (BRL44408 base) were synthesized by Servier (Institut de Recherches Servier, Paris, France) chemists.

**Results**

**Influence of α₂-AR Agonists and Antagonists upon Dialysis Levels of ACh in the FCX of Freely Moving Rats.** In the absence of AChE inhibitors, basal dialysate levels of ACh were 2.18 ± 0.38 pg/20 µl (12 ± 2 fmol/20 µl) (Fig. 1A). As shown in Fig. 2, the injection of vehicle (1 ml/kg) induced a significant, although modest and transient (20 min), increase in extracellular levels of ACh in the FCX. The α₂-AR receptor agonist UK14,304 induced a pronounced and dose-dependent (0.16–2.5 mg/kg s.c.) decrease (maximal effect, −82 ± 3% versus basal values) in ACh levels (Fig. 2), an action mimicked by the preferential α₂A-AR agonist guanabenz (0.16–10.0 mg/kg s.c.) (maximal effect, −69 ± 3% versus basal values), although with a less sustained duration of action (Fig. 3). In contrast, the selective α₂A-AR antagonists atipamezole (0.63–630 µg/kg s.c.) and RX821002 (0.01–2.5 mg/kg s.c.), dose dependently elevated levels of ACh, with peak effects of +168 ± 26 and +130 ± 40%, respectively (Fig. 2). Likewise, the selective α₂A-AR antagonist BRL44408 (2.5–40.0 mg/kg s.c.) markedly elevated levels of ACh (maximal effect, +115 ± 15% versus basal values). In contrast, BRL41992 (10.0 mg/kg s.c.), a preferential α₂B/2C-AR antagonist, and prazosin (10.0 mg/kg s.c.), a preferential antagonist at α₂B/2C-ARs (and a potent α₁-AR antagonist), were inactive (Fig. 3).

**Fig. 1.** Chromatogram showing identification and quantification of acetylcholine. A, 20 µl of standards (1, 5, and 10 pg) of ACh were injected onto the column. Chromatographic Procedures as described under Materials and Methods. The retention time of ACh was 7.8 min. B, 20 µl of a 20-µl basal microdialysis sample plus 10 µl of acetic acid 0.01% was injected onto the column. In a representative, basal, frontocortical dialysate sample, the quantity of ACh was 1.9 pg. The administration of piribedil (10.0 mg/kg s.c.) increased FCX dialysate levels of ACh to a peak of ACh of 5.9 pg.

**Influence of Single Doses of Piribedil, Talipexole, and Quinelorane upon Dialysis Levels of DA Compared with ACh in the FCX of Freely Moving Rats.** In an initial study, we examined the influence of single, equieffective doses of piribedil, talipexole, and quinelorane upon dialysis levels of DA in FCX. Reflecting their agonist properties at D₂/D₃ autoreceptors (Millan et al., 2000), they all elicited marked and significant decreases in frontocortical levels of DA with comparable maximal effects of −49 ± 9, −55 ± 10, and −50 ± 8% versus basal values, respectively (Fig. 4). At these equivalent doses, it can be seen from Fig. 4 that piribedil elicited a pronounced and significant elevation in ACh levels in FCX, whereas talipexole, in an opposite manner, reduced levels of...
ACh; quinelorane did not significantly modify ACh levels. Thus, despite a common, suppressive influence upon DA levels, piribedil, talipexole, and quinelorane differentially modified extracellular levels of ACh in FCX.

Fig. 2. Influence of the α2-AR agonist UK14,304 and of the α2-AR antagonists atipamezole and RX821002 upon dialysis levels of acetylcholine in the frontal cortex of freely moving rats. A, UK14,304. B, atipamezole, and C, RX821002. Data are means ± S.E.M. In the frontal cortex, basal levels of ACh were 2.18 ± 0.38 pg/20 μl. ANOVA data are as follows: UK14,304 (0.16; n = 5) F(1,9) = 2.9, P > 0.05; UK14,304 (0.63; n = 5) F(1,9) = 5.2, P < 0.05; UK14,304 (2.5; n = 5) F(1,9) = 59.4, P < 0.01; atipamezole (0.0063; n = 5) F(1,9) = 0.3, P > 0.05; atipamezole (0.01; n = 6) F(1,10) = 27.6, P < 0.01; and atipamezole (0.03; n = 6) F(1,10) = 6.4, P < 0.05. RX821002 (0.01; n = 6) F(1,10) = 45.9, P < 0.01; and RX821002 (2.5; n = 6) F(1,10) = 5.2, P < 0.05. Asterisks indicate significance of drug-treated versus vehicle-treated (n = 6) values. *, P < 0.05.

Dose-Dependent Influence of Piribedil Compared with Talipexole upon Dialysis Levels of ACh in the FCX of Freely Moving Rats. In subsequent studies, it was found that piribedil elicited a dose-dependent (0.63–40.0 mg/kg s.c.),
pronounced, and sustained increase in dialysis levels of ACh (maximal effect, +219 ± 24% versus basal values) (Fig. 5). In distinction, talipexole provoked a dose-dependent (0.63–10.0 mg/kg s.c.) reduction in extracellular levels of ACh (maximal effect, −79 ± 5% versus basal values) (Fig. 5). After pretreatment with a maximally effective dose of RX821002 (2.5 mg/kg s.c.), piribedil (10.0 mg/kg s.c.) failed to significantly modify levels of ACh. This lack of “additive” or “synergistic” effects indicates that they act at a common site. The inhibitory influence of talipexole (10.0 mg/kg s.c.) upon ACh levels was further “canceled out” by pretreatment with RX821002 (Fig. 5).

Influence of Piribedil Compared with RX821002 and UK14,304 upon Dialysis Levels of ACh in the Dorsal Hippocampus of Freely Moving Rats. Whereas the α2-AR agonist UK14,304 (2.5 mg/kg s.c.) markedly suppressed dialysis levels of ACh in dorsal hippocampus, they were elevated by the α2-AR antagonist RX821002 (2.5 mg/kg s.c.) (maximal effects, −71.9 ± 6.7 and + 122.0 ± 27.0% versus basal values, respectively) (Fig. 6). In analogy to the FCX, piribedil elicited a dose-dependent (2.5–40.0 mg/kg s.c.) and sustained increase in dialysis levels of ACh (maximal effect, +126.7 ± 33.0% versus basal values) (Fig. 6).

Discussion

Technical Considerations: Muscarinic Modulation of Frontocortical Release of ACh. Owing to the high capacity and rapid kinetics of AChE, extracellular levels of ACh are greatly (−1000-fold) exceeded by those of its metabolite, choline. This renders detection of extracellular levels of ACh difficult and has necessitated addition of AChE inhibitors to dialysis perfusates. In contrast, corroborating the work of Ichikawa et al. (2000, 2002), introduction of a supplementary, choline oxydase-loaded, “enzyme-immobilized” column before the analytical column eliminated choline from the chromatogram; thus, the fidelity and sensitivity of
ACh detection was substantially improved. Accordingly, even “resting” levels of ACh could be reproducibly quantified and values of 2.18 ± 0.38 pg/20 μl (15.0 ± 2.6 fmol/20 μl) correspond well to those of Ichikawa et al. (2000, 2002) (19.5 ± 0.7 fmol/20 μl). They are considerably (>20-fold) lower than “basal” levels generated in the presence of AChE inhibitors (Cuadra and Giacobini, 1995; Tellez et al., 1997). Furthermore, the AChE inhibitor, eserine, increased ACh levels by ~7-fold (A. Gobert and M. J. Millan, unpublished observation) in line with its pronounced increase in ACh levels upon local perfusion (Ichikawa et al., 2002). In an extension of the work of Ichikawa et al. (2002), we demonstrate herein that this technique also permits the reliable detection and quantification of ACh levels in dorsal hippocampus. In this structure, basal levels of ACh herein, 1.30 ± 0.16 pg/20 μl (9.0 ± 1.0 fmol/20 μl), were substantially lower than those documented using AChE inhibitors (e.g., 860 fmol/36 μl with 0.3 μM neostigmine; Shirazi-Southall et al., 2002).

Quantification of ACh levels in the absence of AChE inhibitors avoids potentially misleading effects due to pharmacological or metabolic interactions with the drug under study (DeBoer and Abercrombie, 1986; Ichikawa et al., 2000, 2002). Furthermore, inasmuch as ACh exerts a tonic, inhibitory feedback upon its own release via muscarinic autoreceptors (Zhang et al., 2002), an elevation in its levels by inhibition of AChE directly modifies actions of agonists and antagonists at these sites (Toide and Arima, 1989; Liu and Kato, 1994; Ichikawa et al., 2002). In addition, for all drug classes, the apparent magnitude of their actions relative to basal values will be distorted by the use of AChE inhibitors.

By analogy to Ichikawa et al. (2000), in vehicle-treated rats, levels of ACh in FCX were transiently increased relative to basal values. Similarly, levels of ACh in dorsal hippocampus displayed a short-lived increase upon vehicle injection (Shirazi-Southall et al., 2002). These responses reflect arousal and cognitive-attentional factors associated with handling and motor activity (Sarter and Bruno, 2000; Giovannini et al., 2001; Hironaka et al., 2001).

α2-AR Modulation of Frontocortical Release of ACh. The finding that the α2-AR agonist UK14,304 and the α2-AR antagonists atipamezole and RX821002, respectively, suppressed and enhanced frontocortical ACh release demonstrates that α2-ARs exert a tonic, inhibitory influence upon ACh release in the FCX of conscious rats. This observation amplifies findings of in vitro studies (Williams and Reiner, 1993) and in vivo studies using AChE inhibitors (Moroni et al., 1983; Tellez et al., 1997). Furthermore, ACh release was reduced by the preferential α2A-AR agonist guanabenz and accelerated by the selective α2A-AR antagonist BRL44408 (Young et al., 1989; Renouard et al., 1994), suggesting a role for the α2A-AR subtype in this effect. Indeed, prazosin, which displays higher affinity at α2B/2C- versus α2A-ARs (Renouard et al., 1994), did not modify ACh levels, in line with a study of Acquas et al. (1998). This observation was underpinned by the lack of effect of a further preferential antagonist at α2B/2C- versus α2A-ARs, BRL41992 (Young et al., 1989), upon...
Facilitatory Influence of Piribedil upon Frontocortical Levels of ACh. Piribedil, which displays marked antagonist properties at α2A- and α2C-ARs (Millan et al., 2001, 2002; Newman-Tancredi et al., 2002a), provoked a rapid, dose-dependent and sustained increase in extracellular levels of ACh in FCX. There are several possible explanations for this finding.

First, piribedil might interact directly with muscarinic mechanisms. However, it shows negligible affinity for cloned human M2 receptors, other (M1, M3, and M4) muscarinic sites, and for AChE (M. J. Millan, unpublished observation). On structural grounds, it is unlikely that metabolites of piribedil would interact with muscarinic mechanisms: in line with this contention, piribedil does not modify muscarinic responses in vivo (M. J. Millan, unpublished observation). Second, a role of α2 and/or α2ARs might be evoked. However, D2/D3 agonists, such as quinpirole, did not increase ACh release in FCX (Day and Fibiger, 1993). Accordingly, the potent D2/D3 agonist quinolone, which is devoid of affinity for α2-ARs, failed to modify dialysis levels of ACh and several other selective D2/D3 agonists also do not enhance ACh levels (A. Gobert, unpublished observation).

Furthermore, this hypothesis cannot accommodate the opposite facilitatory and inhibitory influence of piribedil and talipexole upon ACh levels, respectively, despite their mutual agonist properties at D2/D3 receptors. Indeed, at doses that elicited an equivalent reduction in FCX release of DA (reflecting activation of D2/D3 autoreceptors), piribedil, talipexole, and quinelorane exerted contrasting influences (increase, decrease, and no change, respectively) upon dialysis levels of ACh (Fig. 4). The doses of RX821002 used herein were shown to block α2A-ARs in previous investigations including, for example, the modulation of frontocortical release of DA and noradrenaline under conditions analogous to the present study of ACh release (Millan et al., 1994; Gobert et al., 1998). Furthermore, the preferential α2B/2C-AR antagonists BRL441992 and prazosin were used at doses previously demonstrated to not block α2A-ARs (Millan et al., 1994; Gobert et al., 1998). However, there is no currently well defined functional model of the role of α2ARs in ACh release. Indeed, in would be of interest to undertake complementary studies in genetically transformed mice lacking (or overexpressing) specific subtypes of α2-AR to corroborate the present observations. Such an approach indicated that α2B- and/or α2C-ARs also, albeit to a minor degree relative to their α2A-AR counterparts, modulate cerebral monoaminergic transmission (Kable et al., 2000; Bücheler et al., 2002).

Fig. 6. Influence of piribedil compared with RX821002 and UK14,304 upon dialysis levels of acetylcholine in the dorsal hippocampus of freely moving rats. A, piribedil, B, RX821002, C, UK14,304. Data are means ± S.E.M. In the dorsal hippocampus, basal levels of ACh were 1.24 ± 0.14 pg/20 μL. ANOVA data are as follows: piribedil (2.5; n = 5) F(1,11) = 2.9, P > 0.05; piribedil (5.0; n = 6) F(1,12) = 5.1, P < 0.05; piribedil (10.0; n = 6) F(1,12) = 22.7, P < 0.01; piribedil (40.0; n = 5) F(1,11) = 21.7, P < 0.01; RX821002 (2.5; n = 5) F(1,11) = 34.0, P < 0.01; and UK14,304 (2.5; n = 6) F(1,12) = 21.7, P < 0.01. Asterisks indicate significance of drug-treated versus vehicle-treated (n = 8) values. *, P < 0.05.

ACh levels. Notably, RX821002 does not interact with imidazoline receptors, which cannot, therefore, be implicated in its induction of ACh release. This pattern of effects resembles studies of frontocortical release of noradrenaline and DA and suggests that α2A-ARs are inhibitory to ACh release (Kable et al., 2000; Millan et al., 2000), consistent with their high density in the FCX and localization on cholinergic cell bodies (Zaborszky et al., 1995; Talley et al., 1996). The doses of BRL441992 used herein were shown to block α2A-ARs in previous investigations including, for example, the modulation of frontocortical release of DA and noradrenaline under conditions analogous to the present study of ACh release (Millan et al., 1994; Gobert et al., 1998). Furthermore, the preferential α2B/2C-AR antagonists BRL441992 and prazosin were used at doses previously demonstrated to not block α2A-ARs (Millan et al., 1994; Gobert et al., 1998). However, there is no currently well defined functional model of the role of cerebral α2B- and/or α2C-ARs appropriate to the precise definition of their active dose ranges at these sites. Thus, it is necessary to be cautious as regards the apparent exclusion of a role of α2B- and/or α2C-ARs in the modulation of ACh release. Indeed, in would be of interest to undertake complementary studies in genetically transformed mice lacking (or overexpressing) specific subtypes of α2-AR to corroborate the present observations. Such an approach indicated that α2B- and/or α2C-ARs also, albeit to a minor degree relative to their α2A-AR counterparts, modulate cerebral monoaminergic transmission (Kable et al., 2000; Bücheler et al., 2002).
shared by talipexole, whereas prazosin (a potent α1-AR antagonist) did not enhance ACh release in FCX.

Thus, in line with above-discussed evidence for a tonic, inhibitory influence of α2-AR heteroceptors upon frontocortical cholinergic transmission, the induction of ACh release in FCX by piribedil likely reflects its antagonist properties at α2-ARs. This interpretation accounts for the opposite suppressive influence of talipexole, an agonist at α2-ARs (Millan et al., 2002; Newman-Tancredi et al., 2002a), upon ACh levels. Moreover, in the presence of a maximally effective dose of RX821002, piribedil failed to elevate ACh levels, indicating a common site of action, whereas the inhibitory influence of talipexole was canceled out by pretreatment with RX821002. Further supporting a role of α2-ARs, the dose range of piribedil that elevated FCX levels of ACh was identical to that which augments frontocortical levels of noradrenaline by blockade of α2-ARs (Millan et al., 2001). Although blockade of the α2A-AR subtype likely participates in the influence of piribedil upon ACh release (vide supra), this issue remains to be directly addressed. Moreover, inasmuch as α2-ARs inhibit ACh release at both the cortical and dendritic level (Moroni et al., 1983; Bertorelli et al., 1991), the precise locus(i) of action of piribedil will require future evaluation.

Facilitatory Influence of Piribedil upon Dorsal Hippocampus Levels of ACh. Although the α2-AR antagonist yohimbine increased extracellular levels of ACh in the ventral hippocampus of rats, it is poorly selective for α2-ARs (Millan et al., 2000) and that study used AChE inhibitors in the dialysate perfusate (Shirazi-Southall et al., 2002). It is thus of interest that using the present procedure, UK14,304 and RX821002, respectively, decreased and enhanced extracellular levels of ACh in the dorsal hippocampus. This observation provides further evidence for a tonic, inhibitory influence of α2-ARs upon ACh release in the dorsal hippocampus, a structure in which their density is particularly high (Talley et al., 1996). Correspondingly, reflecting its antagonist properties at α2-ARs, piribedil dose dependently elevated dialysis levels of ACh in the dorsal hippocampus, a finding paralleling its actions in the FCX.

General Considerations. First, the present study exploited a technique developed by Ichikawa et al. (2000, 2002) in freely moving rats that does not require systemic or local administration of drugs to artificially elevate basal values of ACh. This strategy, analogous to that used for evaluation of extracellular levels of monoamines (Gobert et al., 1998; Millan et al., 2000), should prove invaluable in the characterization of the modulation of cerebral cholinergic transmission by psychotropic agents.

Second, piribedil, via its distinctive antagonist properties at α2-ARs (Millan et al., 2001, 2002), reinforced frontocortical and hippocampal cholinergic transmission. This action may well contribute to its enhancement of cognitive-attentional function (Maurin et al., 2001; Nagaraja and Jayashree, 2001; Smith et al., 2002). Indeed, although behavioral studies are required to underpin this contention, there is preliminary evidence that AChE inhibitors exert a favorable influence upon cognitive function in parkinsonian patients (Reading et al., 2001). Inasmuch as piribedil (like other α2-AR antagonists) also enhances noradrenaline release in FCX (Millan et al., 2001), the relative contribution of cholinergic versus adrenergic mechanisms to its influence upon cognitive-attentional function will be of interest to evaluate.

Third, frontocortical cholinergic pathways also influence motor function, anxiety, sleep and mood (Perry et al., 1999; Sarter and Bruno, 2000; Giovannini et al., 2001; Ichikawa et al., 2002). Thus, a broader exploration of the functional significance of an increase in FCX release of ACh to the management of PD would be justified. Notably, deficits in cholinergic (frontocortical and pedunculopontine) transmission are implicated in the perturbation of sleep and hallucinations experienced by parkinsonian patients (Perry et al., 1999; Sarter and Bruno, 2000). Furthermore, AChE inhibitors have been reported to ameliorate psychotic symptoms in patients in PD (Reading et al., 2001; Bergman and Lerner, 2002).

Finally, although their pronounced side effects (including disruption of sleep and induction of psychosis and cognitive deficits; c.f., paragraphs above) greatly limit their use, muscarinic antagonists have been used in the treatment of PD, principally in the management of refractory tremor and severe L-DOPA-induced dyskinesias (Hurtig, 1997; Wilms et al., 1999; Jenner, 2000; Singer, 2002). These actions do not reflect their blockade of autoreceptors (thereby enhancing ACh release), rather antagonism of postsynaptic sites in the striatum. Furthermore, D2 receptors exert an inhibitory influence upon ACh release in the striatum (Di Chiara et al., 1994; DeBoer and Abercrombie, 1996). In the light of these comments, an interesting question concerns the influence of piribedil compared with other agents upon the striatal release of ACh. In fact, there is no evidence for a role of α2-ARs in the control of striatal cholinergic transmission, so its influence upon ACh release therein should not, in principle, differ from those of talipexole, quinolnolane, or other agents. This remains to be directly demonstrated. In any case, notwithstanding possible benefits of increased corticolimbic release of ACh in the control of cognitive-attentional function (vide supra), such actions would not be expected to markedly modify the motor symptoms of PD per se.

Conclusions. Using an innovative dialysis approach not requiring use of AChE inhibitors, the present study demonstrates that the antiparkinson agent piribedil, which possesses marked antagonist properties at α2-ARs, markedly enhances release of ACh in the FCX and dorsal hippocampus of freely moving rats. These actions may be distinguished to the inhibitory influence of talipexole, which acts as an agonist at α2-ARs, and to the lack of effect of quinolnolane, which does not interact with α2-ARs. A reinforcement of frontocortical cholinergic transmission may contribute to the facilitatory influence of piribedil upon cognitive-attentional function, which is compromised in PD, although it would not be expected to modify motor performance per se. Thus, the present data encourage additional neurochemical, behavioral, and clinical studies of the functional significance of cholinergic transmission and its modulation by α2-ARs to the etiology and management of PD.

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