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PIRIBEDIL ENHANCES FRONTOCORTICAL AND HIPPOCAMPAL RELEASE OF ACETYLCHOLINE IN FREELY-MOVING RATS BY BLOCKADE OF α_{2A}-ADRENOCEPTORS: A DIALYSIS COMPARISON TO TALIPEXOLE AND QUINELORANE IN THE *ABSENCE* OF ACETYLCHOLINESTERASE INHIBITORS

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Running Title : **α_2-adrenoceptors and Parkinson's disease**

Abbreviations :

ACh	Acetylcholine
AChE	Acetylcholinesterase
AR	Adrenoceptor
DA	Dopamine
FCX	Frontal cortex
L-DOPA	L-dihydroxyphenylalanine
PD	Parkinson's disease

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ABSTRACT

In a dialysis procedure *not* requiring perfusate addition of acetylcholinesterase (AChE) 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 to those of other antiparkinson drugs and selective ligands at α_2-adrenoceptors (AR)s. Suggesting a tonic, inhibitory influence of α_{2A}-ARs upon cholinergic transmission, the α_2-AR agonist, UK14,304, and the preferential α_{2A}-AR agonist, guanabenz, reduced levels of ACh, whereas they were elevated by the antagonists, RX821002 and atipamezole, and by the preferential α_{2A}-AR antagonist, BRL44008. In contrast, BRL41992 and prazosin, preferential $\alpha_{2B/2C}$-AR antagonists, were inactive. The dopaminergic agonist and antiparkinson agent, piribedil, which behaves as an antagonist at α_2-ARs, dose-dependently increased extracellular levels of ACh. This action was absent upon pre-treatment with a maximally-effective dose of RX821002. On the other hand, a further dopaminergic agonist and antiparkinson agent, talipexole, which possesses *agonist* properties at α_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* α_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 α_{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.

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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. Though the dopamine (DA) precursor, L-dihydroxyphenylalanine (L-DOPA), is universally employed in the treatment of PD, certain motor symptoms, as well as the accompanying mnesic, sensory and emotional deficits, are little improved (Jenner, 1995). Further, 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. Though 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). While 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 with dopaminergic receptors (see Millan et al., 2002; Newman-Tancredi et al., 2002a, b). Notably, several recognize α_2-ARs. For example, talipexole is an agonist both at D_2/D_3 receptors *and* at α_2-ARs (Meltzer et al., 1989; Millan et al, 2002; Newman-Tancredi et al, 2002a), while piribedil (Jenner, 1995; Smith et al., 2002) behaves as an agonist at D_2/D_3 receptors yet as an *antagonist* at α_{2A}- and α_{2C}-ARs (Millan et al., 2001, 2002; Newman-Tancredi et al., 2002a, b). Further, in distinction to most other antiparkinson agents, both piribedil and talipexole show negligible affinity for serotonergic receptors (Newman-Tancredi et al., 2002b). The distinctive profile of piribedil is of considerable interest inasmuch as α_2-AR antagonists enhance antiparkinson actions of dopaminergic agonists and L-DOPA in rodent and primate models of PD, and suppress the induction of dyskinesias (Brefel-Courbon et al., 1998; Bezard et al., 2001). These actions may reflect blockade of α_{2C}-ARs which are enriched in the striatum (Rosin et al., 1996; Bücheler et al., 2002). They also likely reflect blockade of tonically-active, inhibitory α_{2A}-AR autoreceptors on ascending adrenergic neurones which play an important role in the control of motor behaviour, cognition and mood (Kable et al., 2000; Millan et al., 2000; Chopin et al., 2002). Indeed, degeneration of adrenergic pathways aggravates PD (Sandyk and Iacono, 1990) and, in experimental models, renders subjects more sensitive to dopaminergic neurotoxins (Bing et al., 1994). In line with these observations, like selective α_2-AR antagonists, piribedil reinforces ascending adrenergic transmission (Millan et al., 2001).

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The significance of α_2-AR antagonist properties may not, however, be restricted to an enhancement of adrenergic transmission. The frontal cortex (FCX) receives an intense input from ascending cholinergic projections originating in the nucleus basalis magnocellularis (Amassiri-Teule et al., 1993; Descarries and Umbriaco, 1995). Together with cholinergic pathways innervating the hippocampus, frontocortical cholinergic projections exert a facilitatory influence upon mnesic processes by the engagement of postsynaptic muscarinic and nicotinic receptors (Broersen et al., 1995; Hironaka et al., 2001). By analogy to Alzheimer's disease, reduced activity of ascending cholinergic projections contributes to cognitive deficits and the perturbation of mood in parkinsonian patients (Dubois et al., 1986; Sarter and Bruno, 1998; Perry et al., 1999; Reading et al., 2001). There is ultrastructural evidence that adrenergic and cholinergic projections interact at the terminal level in the cortex and limbic regions (Descarries and Umbriaco, 1995; Li et al., 2001), while adrenergic neurons derived from the locus coeruleus also target cholinergic perikarya (Smiley et al., 1999; Hajszan and Zaborszky, 2002). Correspondingly, α_2-ARs are localized in the FCX, hippocampus and cerebral regions containing cholinergic perikarya (Talley et al., 1996; Rosin et al., 1996). These observations provide an anatomical substrate for functional interactions amongst cholinergic and adrenergic pathways (Cuadra and Giacobini, 1995; Niitykoski et al., 1997) and for neurochemical evidence that α_2-ARs inhibit release of ACh both in the FCX (Moroni et al., 1983; Tellez et al., 1997) and, according to a recent study (Shirazi-Southall et al., 2002), the hippocampus. However, the identity of (the) α_2-AR subtype(s) involved has not been 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 observations, we hypothesized that, in analogy to α_2-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 α_2-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 to the effects of talipexole and of quinelorane, the latter a potent dopaminergic agonist *lacking* affinity at α_2-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, as compared to α_2-AR ligands, upon levels of ACh in the dorsal hippocampus was evaluated.

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METHODS

Animals. Male Wistar rats (Iffa Credo, l'Arbresle, France) of 225-250 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 12h/12h light/dark cycle (lights on at 7.30 a.m.). 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 anaesthesia (60 mg/kg, i.p.). As previously described (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, 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 NaCl (147.2 mM); KCl (4 mM); $CaCl_2$ (2.3 mM), pH 7.3. Two hours after implantation, 20 min dialysate samples were collected for 3 hours. Three basal samples were collected prior to drug administration. In the antagonist studies, RX821002 was injected 20 min prior to 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 µl dialysate samples were collected on 10 µl acetic acid 0.01%. Twenty µl aliquots were then analysed by HPLC. The mobile phase was composed of Na_2HPO_4 (50 mM) and Proclin (BAS, Congleton, UK) (0.5 %), adjusted to pH 8.2 with H_3PO_4. The stationary phase was comprised of a cation ion exchanger (Sepstik, 530 x 1.0 mm, particle size, 10 µm, BAS), a pre-column (pre-immobilised enzyme reactor, 55 x 1 mm) of choline oxydase/catalase (BAS) and a post-column (post-immobilised enzyme reactor, 50 x 1 mm) of choline oxydase/AChE (BAS) maintained at 35°C. An amperometric detector (BAS LC-4B) was used for quantification. The electrode was set at + 100 mV *versus* Ag/AgCl. The glassy carbon electrode (MF2098, BAS) was coated with the peroxydase-redox polymer. The mobile phase was delivered at a flow rate of 0.14 ml/min. The sensitivity of the essay for ACh was 0.1 pg (= 0.55 fmol) (injected in a volume of 20 µl). DA levels were quantified by HPLC followed by coulometric detection as previously (Millan *et al.*, 2001). The assay limit of sensitivity was 0.1 pg/sample. Data were analysed 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, quinelorane 2HCl, RX821002 {2(2-methoxy-1,4 benzodioxan-2-yl)-2-

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imidazoline} HCl, prazosin HCl and talipexole 2HCl were purchased from Sigma (Chesnes, France). Atipamezole HCl, piribedil monomethane sulfonate (Trivastal®), UK14,304 {5-bromo-6-[2-imidazolin-2-yl-amino]-quinoxaline} tartrate, BRL41992 maleate {trans-2,3,9,13b-tetrahydro-1,2-dimethyl-1H-dibenz[c,f]imidazo[1,5-a]azepine} and BRL44408 base {2-(2H-(1-methyl-1,3-dihydroisoindole)methyl)-4,5-dihydroimidazole} were synthesized by Servier chemists.

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RESULTS

Influence of α_2-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 fmoles/20µl) (Fig. 1). As shown in Figure 2, the injection of vehicle (1 ml/kg) induced a significant, though modest and transient (20 min), increase in extracellular levels of ACh in the FCX. The α_2-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 α_{2A}-AR agonist, guanabenz (0.16-10.0 mg/kg, s.c.) (maximal effect, -69 ± 3 % *versus* basal values), though with a less sustained duration of action (Fig. 3). In contrast, the selective α_2-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 α_{2A}-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 $\alpha_{2B/2C}$-AR antagonist and prazosin (10.0 mg/kg, s.c.), a preferential antagonist at $\alpha_{2B/2C}$-ARs (and a potent α_1-AR antagonist), were inactive (Fig. 3).

Influence of single doses of piribedil, talipexole and quinelorane upon dialysis levels of DA as compared to ACh in the FCX of freely-moving rats. In an initial study, we examined the influence of single, equi-effective doses of piribedil, talipexole and quinelorane upon dialysis levels of DA in FCX. Reflecting their agonist properties at D_2/D_3 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 fashion, 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.

Dose-dependent influence of piribedil as compared to 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). Following pre-treatment with a maximally-effective dose of RX821002 (2.5 mg/kg, s.c.),

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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, "cancelled out" by pre-treatment with RX821002 (Fig. 5).

Influence of piribedil as compared to RX821002 and UK14,304 upon dialysis levels of ACh in the dorsal hippocampus of freely-moving rats. While the α_2-AR agonist, UK14,304 (2.5 mg/kg, s.c.), markedly suppressed dialysis levels of ACh in dorsal hippocampus, they were elevated by the α_2-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).

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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. By contrast, corroborating the work of Ichikawa et al. (2000, 2002), introduction of a supplementary, choline oxydase-loaded, "enzyme-immobilized" column *prior* to 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/20µl (15.0 ± 2.6 fmol/20µl) correspond well to those of Ichikawa et al. (2000, 2002) (19.5 ± 0.7 fmol/20µl). They are considerably (> 20-fold) lower than "basal" levels generated in the presence of AChE inhibitors (e.g., Cuadra and Giacobini, 1995; Tellez et al., 1997). Further, the AChE inhibitor, eserine, increased ACh levels by ~7-fold (unpub. obs.) 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), moreover, we demonstrate herein that this technique also permits the reliable detection and quantitation of ACh levels in dorsal hippocampus. In this structure, basal levels of ACh herein, 1.30 ± 0.16 pg/20µl (9.0 ± 1.0 fmol/20µl) were substantially lower than those documented employing AChE inhibitors (e.g., 860 fmol/36µl 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, 1996; Ichikawa et al., 2000, 2002). Further, 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).

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Alpha$_2$-AR modulation of frontocortical release of ACh. The finding that the α_2-AR agonist, UK14,304, and the α_2-AR antagonists, atipamezole and RX821002, respectively suppressed and enhanced frontocortical ACh release demonstrates that α_2-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 employing AChE inhibitors (Moroni et al., 1983; Tellez et al., 1997). Further, ACh release was reduced by the preferential α_{2A}-AR agonist, guanabenz, and accelerated by the selective α_{2A}-AR antagonist, BRL44408 (Young et al., 1989; Renouard et al., 1994) suggesting a role for the α_{2A}-AR subtype in this effect. Indeed, prazosin, which displays higher affinity at $\alpha_{2B/2C}$- *versus* α_{2A}-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 $\alpha_{2B/2C}$- *versus* α_{2A}-ARs, BRL41992 (Young et al., 1989), upon 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 α_{2A}-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 BRL44408 employed herein were shown to block α_{2A}-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). Further, the preferential $\alpha_{2B/2C}$-AR antagonists, BRL41992 and prazosin, were employed herein at doses previously demonstrated *not* to block α_{2A}-ARs (Millan et al., 1994; Gobert et al., 1998). However, there is no currently well-defined functional model of the role of cerebral α_{2B}- and/or α_{2C}-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 α_{2B}- and/or α_{2C}-ARs in the modulation of ACh release. Indeed, in would be of interest to undertake complementary studies in genetically-transformed mice lacking (or over-expressing) specific subtypes of α_2-AR in order to corroborate the present observations. Such an approach indicated that α_{2C}-ARs also - albeit to a minor degree relative to their α_{2A}-AR counterparts - modulate cerebral monoaminergic transmission (Kable et al., 2000; Bücheler et al., 2002).

Facilitatory influence of piribedil upon frontocortical levels of ACh. Piribedil, which displays marked antagonist properties at α_{2A}- and α_{2C}-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.

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First, piribedil might interact directly with muscarinic mechanisms. However, it shows negligible affinity for cloned, human M_2 receptors, other (M_1, M_3 and M_4) muscarinic sites and for AChE (Millan MJ, unpub. obs.). 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* (Millan MJ, unpub. obs.). *Second*, a role of D_2 and/or D_3 receptors might be evoked. However, D_2/D_3 agonists, such as quinpirole, did *not* increase ACh release in FCX in a previous study (Day and Fibiger, 1993). Accordingly, the potent D_2/D_3 agonist, quinelorane, which is *devoid* of affinity for α_2-ARs, *failed* to modify dialysis levels of ACh and several other selective D_2/D_3 agonists also do not enhance ACh levels (Gobert A, unpub. obs.). Further, this hypothesis cannot accommodate the opposite facilitatory and inhibitory influence of piribedil and talipexole upon ACh levels, respectively, despite their mutual agonist properties at D_2/D_3 receptors. Indeed, at doses which elicited an equivalent *reduction* in FCX release of DA (reflecting activation of D_2/D_3 autoreceptors), piribedil, talipexole and quinelorane exerted contrasting influences (increase, decrease and no change, respectively) upon dialysis levels of ACh (Fig. 4). *Third*, the weak antagonist properties of piribedil at α_1-ARs (Millan et al., 2002; Newman-Tancredi et al., 2002a) are unlikely to be implicated since they are shared by talipexole, while prazosin, (a potent α_1-AR antagonist) did not enhance ACh release in FCX.

Thus, in line with above-discussed evidence for a tonic, inhibitory influence of α_2-AR heteroceptors upon frontocortical cholinergic transmission, the induction of ACh release in FCX by piribedil likely reflects its antagonist properties at α_2-ARs. This interpretation accounts for the opposite suppressive influence of talipexole, an agonist at α_2-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, while the inhibitory influence of talipexole was "cancelled out" by pre-treatment with RX821002. Further supporting a role of α_2-ARs, the dose-range of piribedil which elevated FCX levels of ACh was identical to that which augments frontocortical levels of noradrenaline by blockade of α_{2A}-ARs (Millan et al., 2001). Though blockade of the α_{2A}-AR subtype likely participates in the influence of piribedil upon ACh release (*vide supra*), this issue remains to be directly addressed. Moreover, inasmuch as α_2-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. Though the α_2-AR antagonist, yohimbine, increased extracellular levels of ACh in the ventral hippocampus of rats, it is poorly selective for α_2-ARs (Millan et al., 2000) and that study employed AChE inhibitors in the dialysate perfusate (Shirazi-Southall et al., 2002). It is, thus,

of interest that, employing 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 α_2-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 α_2-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 which does not require systemic or local administration of drugs to artificially elevate basal values of ACh. This strategy, analogous to that employed 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 α_2-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, though behavioural studies are required to underpin this contention, there is preliminary evidence that AChE inhibitors exert a favourable influence upon cognitive function in parkinsonian patients (Reading et al., 2001). Inasmuch as piribedil (like other α_2-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 pedonculopontine) transmission are implicated in the perturbation of sleep and hallucinations experienced by parkinsonian patients (Perry et al., 1999; Sarter and Bruno, 2000). Further, AChE inhibitors have been reported to ameliorate psychotic symptoms in patients in PD (Reading et al., 2001; Bergman and Lerner, 2002).

Finally, though their pronounced side-effects (including disruption of sleep and induction of psychosis and cognitive deficits – c.f. above paragraphs) greatly limit their use, muscarinic

antagonists have been employed in the treatment of PD, principally in the management of refractory tremor and severe L-DOPA-induced dyskinesias (Wilms et al., 1999; Hurtig, 1997; 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. Further, D_2 receptors exert an inhibitory influence upon ACh release in the striatum (DeBoer and Abercrombie, 1996; Di Chiara et al., 1994). In the light of these comments, an interesting question concerns the influence of piribedil as compared to other agents upon the striatal release of ACh. In fact, there is *no* evidence for a role of α_2-ARs in the control of striatal cholinergic transmission, so its influence upon ACh release therein should *not*, in principle, differ from those of talipexole, quinelorane 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. Employing 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 α_2-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 α_2-ARs, and to the lack of effect of quinelorane, which does not interact with α_2-ARs. A reinforcement of frontocortical cholinergic transmission may contribute to the facilitatory influence of piribedil upon cognitive-attentional function, which is compromized in PD, though it would not be expected to modify motor performance *per se*. Thus, the present data encourage additional neurochemical, behavioural and clinical studies of the functional significance of cholinergic transmission and its modulation by α_2-ARs to the aetiology and management of PD.

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Table 1. Summary of drug pharmacological profiles

DRUG	ACTIVITY	REFERENCE
UK14,304	α_2-AR agonist	Renouard et al., 1994
Atipamezole	α_2-AR antagonist	Renouard et al., 1994
RX821002	α_2-AR antagonist	Renouard et al., 1994
Guanabenz	Preferential α_{2A}-AR agonist	Renouard et al., 1994
BRL44408	Preferential α_{2A}-AR antagonist	Young et al., 1989
BRL41992	Preferential α_{2B}-AR antagonist	Young et al., 1989
Prazosin	Preferential $\alpha_{2B/2C}$-AR (and α_1-AR) antagonist	Renouard et al., 1994
Piribedil	α_2-AR antagonist and D_2 agonist	Millan et al., 2002
Talipexole	α_2-AR agonist and D_2 agonist	Millan et al., 2002
Quinelorane	D_2 agonist	Millan et al., 2002

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Figure 1. Chromatogram showing identification and quantification of acetylcholine.

Panel A: 20 µl of standards (1, 5, 10 pg) of ACh were injected onto the column employing chromatographic conditions as described in Methods. The retention time of ACh was 7.8 min. Panel B: 20 µl of a 20 µl basal microdialysis sample plus 10 µl 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.

Figure 2. Influence of the α_2-AR agonist, UK14,304, and of the α_2-AR antagonists, atipamezole and RX821002, upon dialysis levels of acetylcholine in the frontal cortex of freely-moving rats.

Panel A, UK14,304; Panel B, Atipamezole and Panel C, RX821002. Data are means ± S.E.M.s. In the frontal cortex, basal levels of ACh were 2.18 ± 0.38 pg/20µl. ANOVA 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 (0.63; N = 5), $F(1,9) = 59.4$, $P < 0.01$; atipamezole (0.00063; N = 5), $F(1,9) = 0.3$, $P > 0.05$; atipamezole (0.01, N = 6), $F(1,10) = 2.7$, $P > 0.05$; atipamezole (0.16; N = 6), $F(1,10) = 19.8$, $P < 0.01$; atipamezole (0.63; N = 6), $F(1,10) = 27.6$, $P < 0.01$; RX821002 (0.01; N = 5), $F(1,9) = 0.1$, $P > 0.05$; RX821002 (0.04; N = 5), $F(1,9) = 6.4$, $P < 0.05$; RX821002 (0.16; 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. * $P < 0.05$.

Figure 3. Influence of the α_{2A}-AR agonist, guanabenz, of the α_{2A}-AR antagonist, BRL44408, and of the preferential $\alpha_{2B/2C}$-AR antagonists, BRL41992 and prazosin, upon dialysis levels of acetylcholine in the frontal cortex of freely-moving rats.

Panel A, Guanabenz; Panel B, BRL44408 and Panel C, Prazosin. Data are means ± S.E.M.s. ANOVA as follows. Guanabenz (0.16; N = 7), $F(1,11) = 0.4$, $P > 0.05$; guanabenz (2.5; N = 6), $F(1,10) = 11.9$, $P < 0.01$; guanabenz (10.0; N = 5), $F(1,9) = 43.4$, $P < 0.01$; BRL44408 (2.5; N = 5), $F(1,9) = 0.6$, $P > 0.05$; BRL44408 (10.0; N = 6), $F(1,10) = 5.7$, $P < 0.05$; BRL44408 (40.0; N = 7), $F(1,11) = 28.3$, $P < 0.01$; prazosin (10.0; N = 6), $F(1,10) = 0.3$, $P > 0.05$ and BRL41992 (10.0; N = 5), $F(1,9) = 0.1$, $P > 0.05$. Asterisks indicate significance of drug-treated *versus* vehicle-treated (N = 6) values. * $P < 0.05$.

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Figure 4. Influence of piribedil, talipexole and quinelorane upon dialysis levels of dopamine as compared to acetylcholine levels in the frontal cortex of freely-moving rats.

Panels A and D, Piribedil; Panels B and E, Talipexole and Panels C and F, Quinelorane. Frontocortical basal levels of dopamine were 1.1 ± 0.3 pg/20µl (Right panels). Data are means ± S.E.M.s. For DA, ANOVA as follows. Piribedil (5.0; N = 5), $F(1,8) = 34.4$, $P < 0.01$; talipexole (2.5; N = 6), $F(1,9) = 32.7$, $P < 0.01$ and quinelorane (0.16; N = 6), $F(1,9) = 70.8$, $P < 0.01$. Asterisks indicate significance of drug-treated *versus* vehicle-treated (N = 5) values. * $P < 0.05$. For ACh, piribedil (5.0; N = 5), $F(1,9) = 9.2$, $P < 0.05$; talipexole (2.5; N = 6), $F(1,10) = 24.0$, $P < 0.01$ and quinelorane (0.16; N = 6), $F(1,10) = 0.8$, $P > 0.05$. Asterisks indicate significance of drug-treated *versus* vehicle-treated (N = 6) values. * $P < 0.05$.

Figure 5. Influence of piribedil as compared to talipexole upon dialysis levels of acetylcholine in the frontal cortex of freely-moving rats: dose-response relationships and influence of pre-treatment with RX821002.

Panel A, Piribedil; Panel B, Talipexole, Panel C, Piribedil following pre-treatment with RX821002 and Panel D, Talipexole following pre-treatment with RX821002. Data are means ± S.E.M.s. ANOVA as follows. Panels A and B, Piribedil (0.63; N = 5), $F(1,9) = 0.1$, $P > 0.05$; piribedil (2.5; N = 5), $F(1,9) = 10.7$, $P < 0.01$; piribedil (10.0; N = 7), $F(1,11) = 6.6$, $P < 0.05$; piribedil (40.0; N = 5), $F(1,9) = 86.8$, $P < 0.01$; talipexole (0.63; N = 5), $F(1,9) = 2.0$, $P > 0.05$ and talipexole (10.0; N = 6), $F(1,10) = 90.6$, $P < 0.01$. Asterisks indicate significance of drug-treated *versus* vehicle-treated (N = 6) values. * $P < 0.05$. Panels C and D, influence of RX821002 (N = 8), $F(1,12) = 9.9$, $P < 0.01$; influence of piribedil (N = 6), $F(1,10) = 32.8$, $P < 0.01$ and interaction (N = 8), $F(1,12) = 0.1$, $P > 0.05$. Influence of RX821002 (N = 8), $F(1,12) = 9.9$, $P < 0.01$; influence of talipexole (N = 6), $F(1,10) = 73.7$, $P < 0.01$ and interaction (N = 5), $F(1,9) = 31.7$, $P < 0.01$. Asterisks indicate significance of drug-treated *versus* vehicle/vehicle-treated (N = 6) values. * $P < 0.05$.

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Figure 6. Influence of piribedil as compared to RX821002 and UK14,304 upon dialysis levels of acetylcholine in the dorsal hippocampus of freely-moving rats.

Panel A, piribedil; Panel B, RX821002 and Panel C, UK14,304. Data are means ± S.E.M.s. In the dorsal hippocampus, basal levels of ACh were 1.24 ± 0.14 pg/20µl. ANOVA 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. * $P < 0.05$.

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Index Terms: Acetylcholine, cognition, dopamine, frontocortical, antiparkinson, Parkinson's disease, dialysis, α_2-ARs.

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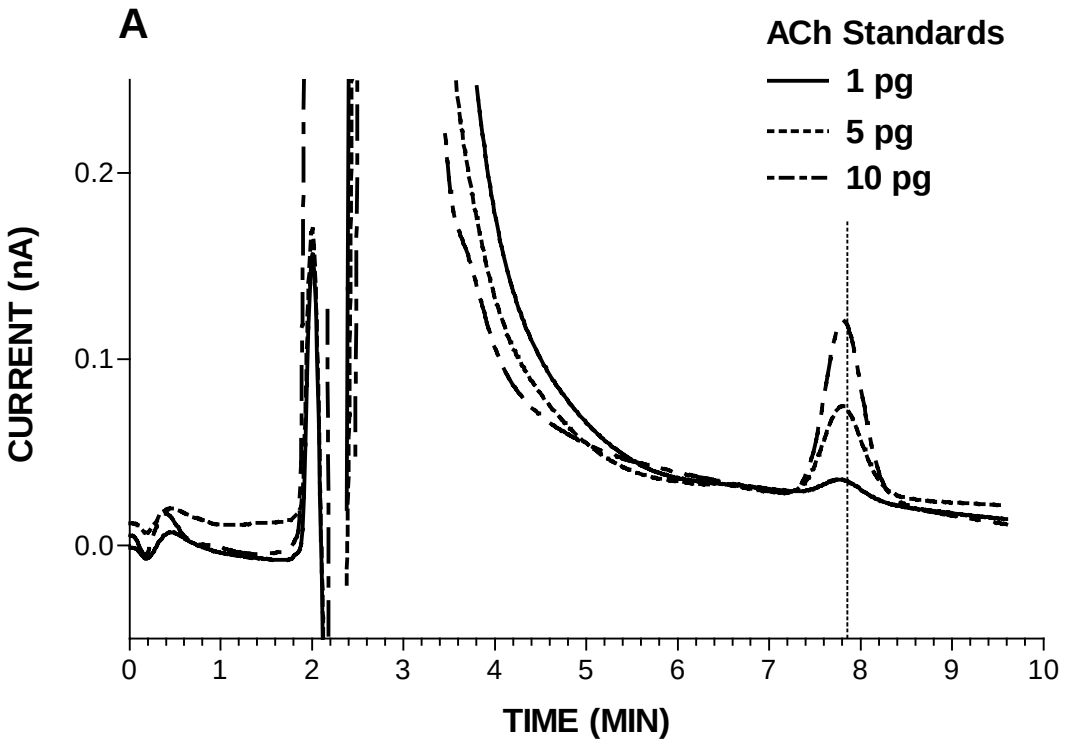

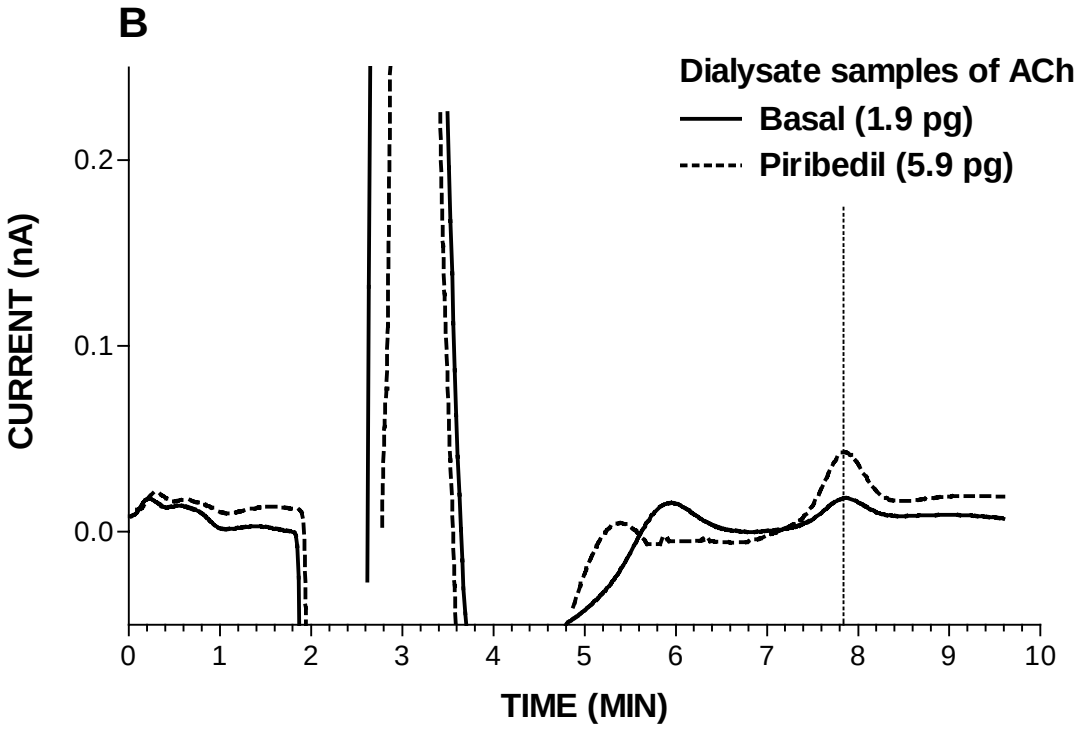

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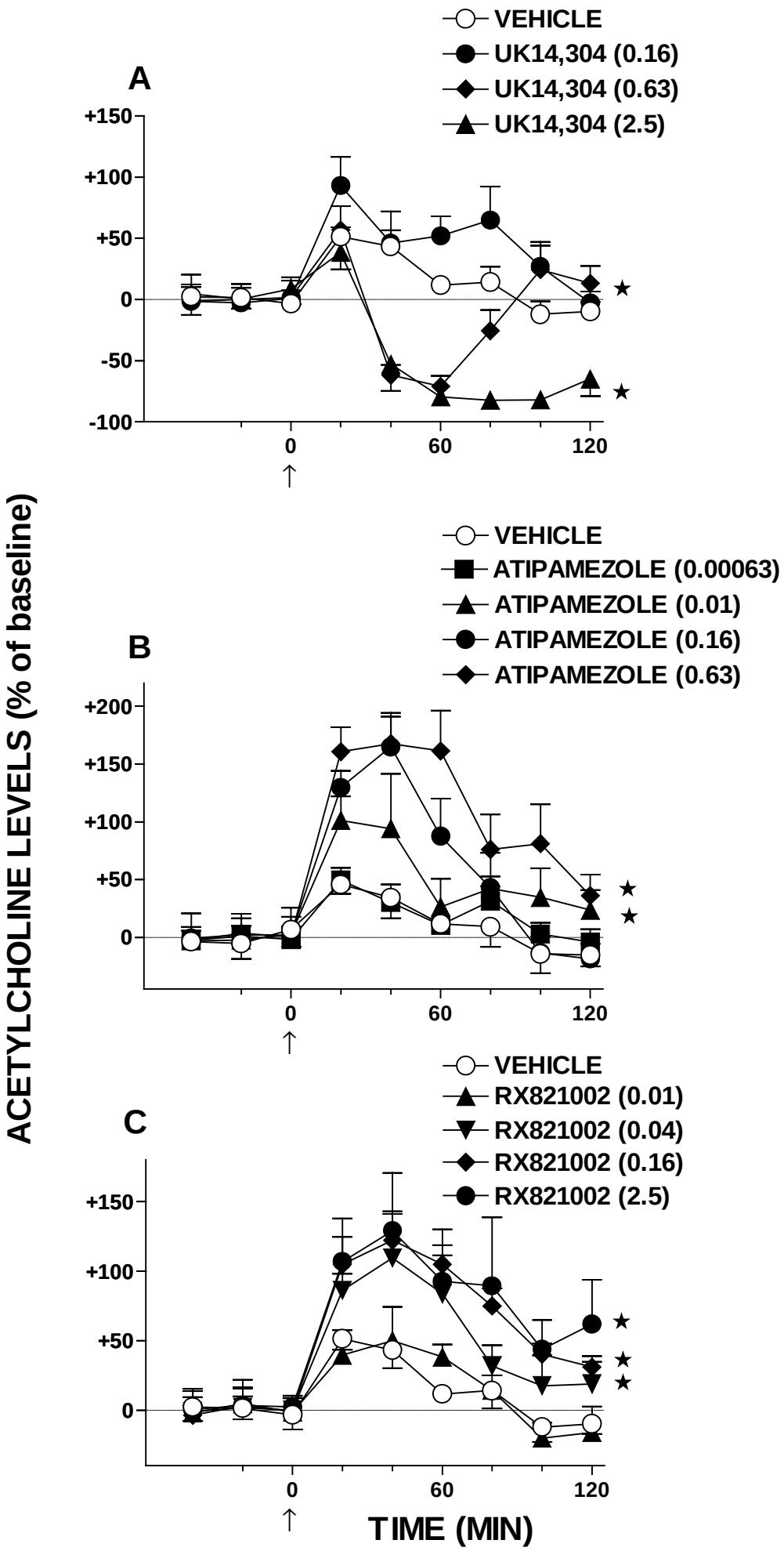

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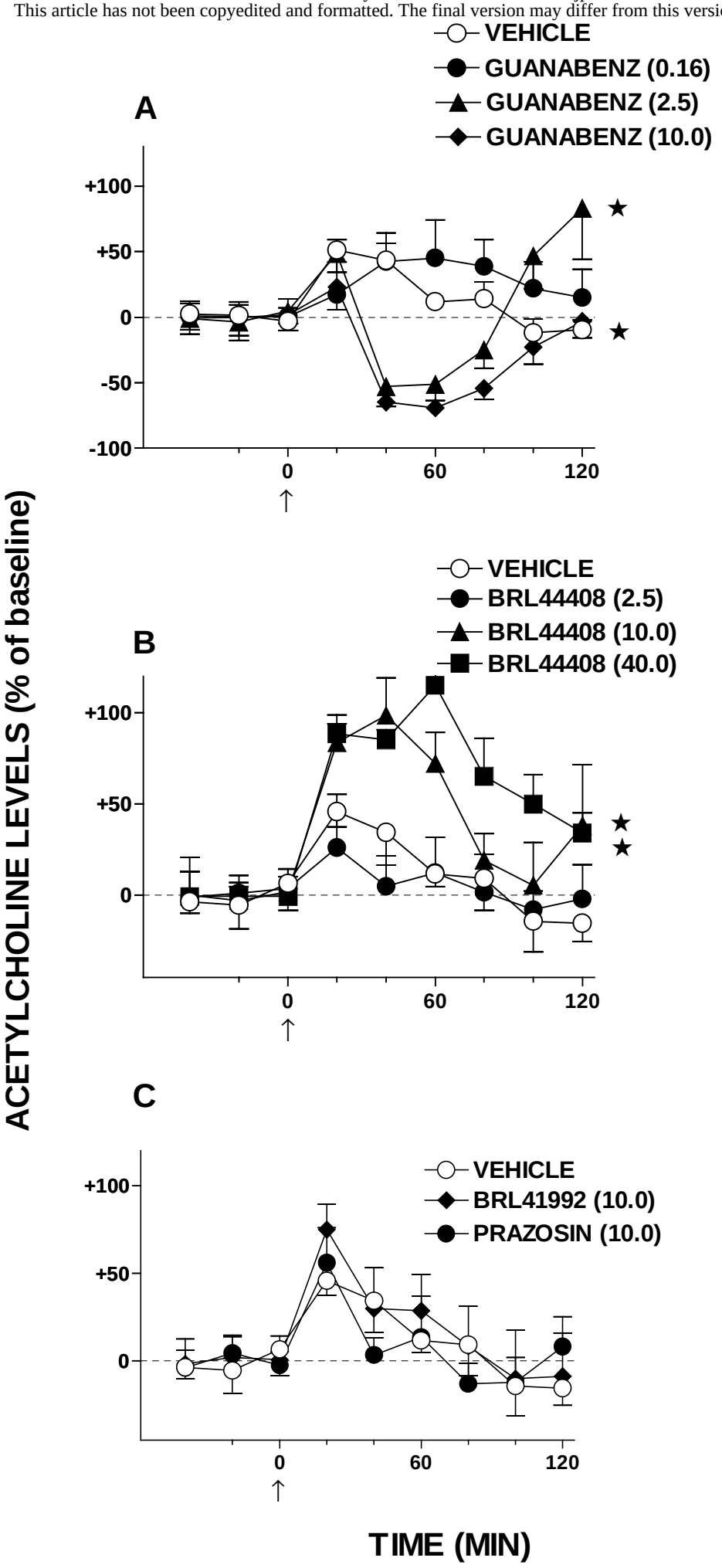

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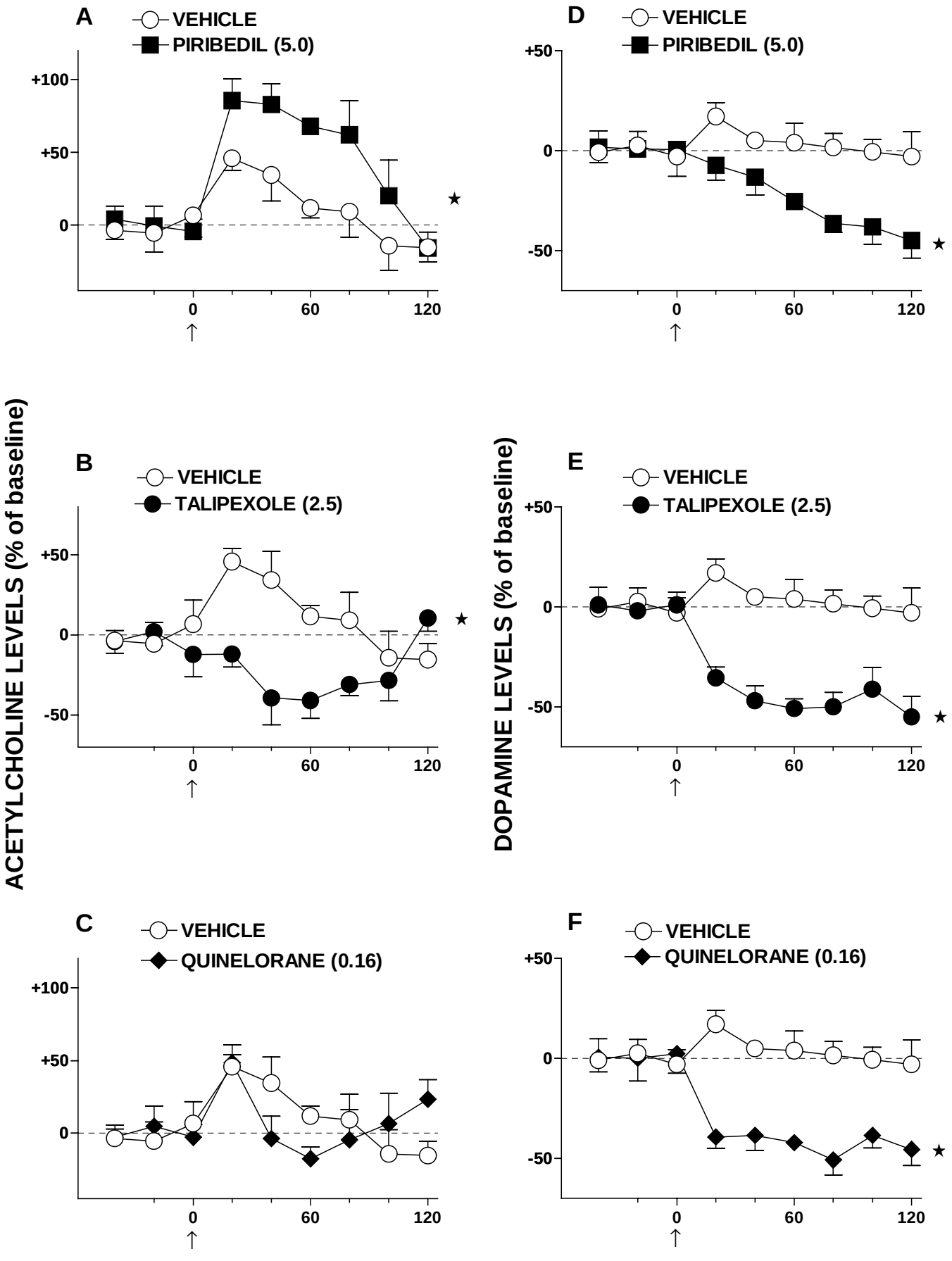

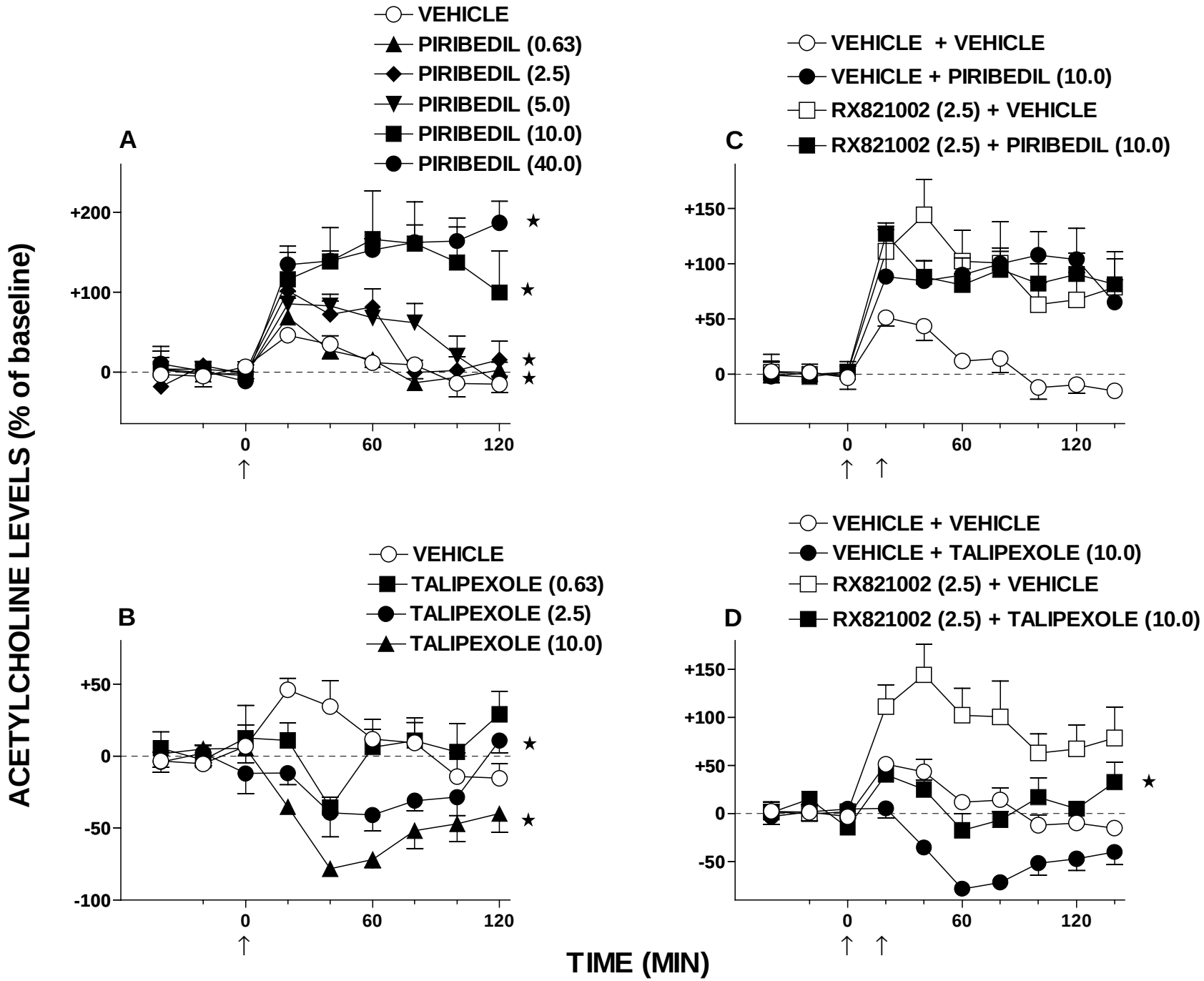

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