Effects of Chlorpyrifos on High-Affinity Choline Uptake and \([^3]H\)Hemicholinium-3 Binding in Rat Brain

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High, subcutaneous doses of the organophosphorus insecticide chlorpyrifos (CPF) in adult male rats can be well-tolerated despite extensive and persistent acetylcholinesterase (AChE) inhibition. We propose that changes in acetylcholine synthesis could modulate the toxicity associated with extensive AChE inhibition following CPF exposure. High-affinity choline uptake (HACU, the rate-limiting step in acetylcholine synthesis) and binding to \([^3]H\)hemicholinium-3 (HC-3, a specific ligand for the choline transporter) were chosen as indicators of acetylcholine synthesis. Female, Sprague-Dawley rats (220-280 g) were treated with either vehicle (peanut oil, 2 ml/kg, sc) or CPF (280 mg/kg, 2 ml/kg, sc), examined daily for clinical signs of toxicity, and sacrificed 1, 2, or 7 days later for neurochemical measurements (AChE inhibition, muscarinic receptor binding using \([^3]H\)quinuclidinyl benzilate (QNB) and \([^3]H\)cis-methyldioxolane (CD) as ligands, HACU and \([^3]H\)HC-3 binding) in frontal cortex. Despite extensive AChE inhibition (90-93%) at all time points, relatively minor degrees of overt toxicity were noted in CPF-treated rats. Binding to the non-selective muscarinic antagonist \([^3]H\)QNB was reduced (10-34%), whereas binding to the putative m2-selective agonist \([^3]H\)CD was increased (15-23%) at all three time points. HACU was reduced (20%) in crude synaptosomes prepared from CPF-treated rats 1 day following exposure but no significant changes were noted at 2 or 7 days after treatment. CPF-oxon, the active oxidative metabolite of CPF, was a weak inhibitor of HACU in vitro (IC50 > 200 μM). Binding to \([^3]H\)HC-3 was reduced in a dose-related manner 1 day after CPF exposure. Kinetic analyses of \([^3]H\)HC-3 binding 1 day after CPF (280 mg/kg) indicated a significant reduction in density (Bmax: control, 187 ± 18 fmol/mg protein; CPF, 104 ± 12 fmol/mg protein) with no apparent change in binding affinity (Kd: control, 25 ± 3 nM; CPF, 19 ± 3 nM). These results suggest that a reduction in HACU/acyetylcholine synthesis may contribute, along with compensatory changes in cholinergic receptors, to the diminished toxicity following extensive AChE inhibition by CPF.

Chlorpyrifos (CPF) is a diethyl-organophosphorothioate insecticide widely used in the United States (Marquis, 1986). As with other organophosphorus (OP) pesticides, CPF exerts acute neurotoxicity primarily through inhibition of acetylcholinesterase (AChE, EC 3.1.1.7) at cholinergic synapses of both the central and the peripheral nervous systems (Ecobichon, 1991). Extensive inhibition of AChE activity leads to accumulation of acetylcholine within the synapse and overstimulation of postsynaptic cholinergic receptors (both muscarinic and nicotinic), which in turn elicits cholinergic toxicity. Characteristic signs of AChE inhibition include autonomic dysfunction (e.g., excessive salivation, lacrimation, urination, and defecation, abbreviated as SLUD syndrome), involuntary movements (e.g., tremors and convulsions), hypothermia, respiratory dysfunction, and a host of others.

Previous studies in our laboratory (Pope et al., 1991, 1992; Chaudhuri et al., 1993) and others (Bushnell et al., 1993; Padilla et al., 1994) have demonstrated that high subcutaneous doses of CPF (up to 280 mg/kg) in adult male rats can cause extensive AChE inhibition but only minimal signs of toxicity. While compensatory downregulation of postsynaptic cholinergic receptors is considered a primary mechanism of tolerance to AChE inhibitors (Costa et al., 1982; Russell and Overstreet, 1987), compensatory presynaptic processes may also participate in the ultimate expression of toxicity with CPF. It has been reported that a subpopulation of muscarinic receptors selectively labeled by \([^3]H\)cis-methyldioxolane (CD) is primarily of the m2-subtype (Huff and AbouDonia, 1994) and located mainly on presynaptic terminals (Watson et al., 1986). These \([^3]H\)CD-labeled receptors have been shown to be sensitive to direct binding to OP compounds such as paraoxon (Jett et al., 1992; Ward et al., 1993) and CPF-oxon (Huff et al., 1994) and may act as autoreceptors regulating acetylcholine release via a feedback inhibitory mechanism (Pope et al., 1995). Changes in these receptors following CPF exposure could therefore regulate the expression of toxicity through modifying the amount of acetylcholine which accumulates within the synapse following extensive AChE inhibition (Pope et al., 1995).

Acetylcholine synthesis is another cholinergic presynaptic process that could be modified by CPF and participate in the expression of toxicity that accompanies extensive AChE inhibition. Lim and co-workers (1987) reported that high-affinity choline uptake (HACU, the rate-limiting step in ace-
tylcholine synthesis) was reduced in rat striatum and hippocampus after subacute exposure to diisopropylfluorophosphate (DFP). Soman and sarin have also been shown to decrease HACU in the cortex and hippocampus from 0.5 to 4 hr after exposure (Whalley and Shih, 1989). In addition, HACU was reduced within 20 min following exposure to dimethylchlorovinylphosphatase (DDVP) (Kobayashi et al., 1986). If acetylcholine synthesis is reduced following OP exposure, fewer acetylcholine molecules may be released into the synapse following stimulation, with a subsequent lessening of the consequences of extensive AChE inhibition. We propose that changes in HACU/acetylcholine synthesis may contribute to the diminished toxicity noted following high subcutaneous doses of this pesticide.

Rather large sex-related differences in response can occur with exposure to some OPs by various routes of exposure (Benke and Murphy, 1975; Sultatos, 1991; Zhang and Sultatos, 1991; unpublished observations). Sultatos (1991) reported that female rats were markedly more sensitive than males to acute toxicity from intraperitoneal chlorpyrifos challenge but little is known regarding possible sex differences in response to high-dose, subcutaneous exposure. The present study was designed to examine the effects of high-dose subcutaneous OP exposure in female rats and determine if high-affinity choline transport was altered following exposure to this toxicant. The in vitro effect of CPF-oxon, the active metabolite of CPF, on HACU was compared to that of hemicholinium-3 (HC-3), a well-known inhibitor of choline transport. Binding to [3H]HC-3, a specific ligand for the high-affinity choline transporter, was also examined to characterize further the changes in choline transport. The results suggest that changes in HACU/acetylcholine synthesis, along with compensatory changes in cholinergic receptors, may play a role in the diminished toxicity noted following high doses of this anticholinesterase.

**METHODS**

**Materials.** Chlorpyrifos (99% purity by IR, TLC, and GC/FID) was purchased from Chem Service (West Chester, PA) and maintained in a dessicator under nitrogen at 4°C. Choline chloride [methyl-3H], specific activity, 81.0 Ci/mmol; hemicholinium-3 diacetate salt [methyl-3H], specific activity, 124.7 Ci/mmol; quinclidinyl benzilate, l-[benzilic-4,4'-3H] (QNB), specific activity, 36.4 Ci/mmol; dioxolane, l (+)-cis-[2-methyl-3H] (CD), specific activity, 64.5 Ci/mmol; and acetylcholine iodide [acetyl-3H], specific activity, 73.7 Ci/mmol, were purchased from Dupont New England Nuclear (Boston, MA). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

**Animals and treatments.** Female Sprague–Dawley rats (3 months old; body weight, 220–280 g; N = 186) were used throughout. Rats were maintained with a 12 hr:12 hr light:dark illumination cycle and housed in individual steel mesh cages for at least 7 days prior to dosing. All procedures involving animals were in accordance with protocols established in the NIH/NRC Guide for the Care and Use of Laboratory Animals and were reviewed by the Institutional Animal Care and Use Committee at Northeast Louisiana University. Animals were subcutaneously treated (along the mid-line of the back) with either vehicle (peanut oil, 2 ml/kg) or CPF (280 mg/kg, 2 ml/kg) and examined daily for clinical signs of acute toxicity for 7 days or sacrificed at 1, 2, or 7 days after treatment for neurochemical measurements (AChE activity, muscarinic receptor binding, HACU and [3H]HC-3 binding). Rats treated with CPF (70, 140, or 280 mg/kg, sc) were sacrificed 1 day after exposure for [3H]HC-3 binding, and saturation analysis was carried out in tissues from rats treated with the highest dosage (280 mg/kg).

**Clinical signs.** Signs of acute neurotoxicity were recorded in two categories. Scores were scaled based on the method of Moser and co-workers (1988) with modifications. All clinical signs were scored by a “blind” observer.

Autonomic dysfunction (SLUD syndrome, an acronym standing for salivation, lacrimation, urination, and defecation) was scored as follows: 1, normal; 2, slight—one sign or very mild multiple SLUD signs; 3, moderate—multiple signs; 4, severe—multiple, extensive SLUD signs.

**Tissue preparation.** On Day 1, 2, or 7 after treatment, rats were decapitated and the frontal cortex was dissected essentially as described by Glowinski and Iversen (1966). Tissue samples were either used fresh (for choline uptake studies) or frozen at −70°C until time of assay. For enzyme and ligand binding studies, homogenates (1:30, w/v, for AChE and QNB, 1:10, w/v, for CD) of frozen/thawed samples were prepared on ice with a Polytron (Brinkmann Instruments, Westbury, NY) in 50 mM Tris(hydroxymethyl)aminomethane buffer, pH 7.4 (25°C), containing the following salts: NaCl, 120 mM; KCl, 5 mM; CaCl2, 2 mM; MgCl2, 1 mM (Tris–salts buffer). These homogenates were then centrifuged at 48,000 g for 10 min to prepare a particulate fraction. The membranous pellets were washed twice by rehomogenization in equal volumes of fresh Tris–salts buffer followed by centrifugation as before. For [3H]HC-3 binding, homogenates (1:50, w/v) of frozen/thawed samples were prepared in 50 mM glycol-glycine buffer, pH 7.8, containing 200 mM NaCl and membranes prepared as above. For choline uptake studies, homogenates (1:29) of fresh tissues were prepared with a Potter–Elvehjem homogenizer in 0.32 M sucrose followed by centrifugation at 1000 g for 10 min. The supernatant was transferred to a clean tube and then centrifuged for 20 min at 17,000 g. The supernatant from this step was discarded and fresh, oxygenated 40 mM Tris(hydroxymethyl)aminomethane buffer, pH 7.5 (25°C), containing 125 mM NaCl, 4.2 mM MgSO4, 9.6 mM KCl, 2.4 mM CaCl2, and 10 mM dextrose was added to the pellet (original volume). The pellet was then resuspended by homogenization as before and this crude synaptosomal preparation was used to measure choline uptake.

**AChE activity.** Enzyme activity was measured radiometrically essentially by the method of Johnson and Russell (1975) using a final concentration of 0.12 mM [3H]acetylcholine iodide. Preliminary experiments delineated conditions of incubation time and tissue concentration necessary for linear rates of substrate hydrolysis. The crude synaptosomal preparations from choline uptake studies and the washed membrane fractions from binding studies were used for assay and enzyme activity was related to protein concentration (Lowry et al., 1951).

**Muscarinic receptor binding.** Total muscarinic receptor ([3H]QNB) binding was measured in washed membranes essentially by the method of Yamamura and Snyder (1974). Binding of [3H]QNB (0.75 nM final concentration) was determined by incubation with 100–200 μg of membrane protein in 50 mM Tris–salts buffer, pH 7.4, for 60 min at 37°C in a final volume of 2 ml. Subsequent to incubation, the samples were vacuum filtered and washed (3 ml, 3×) over Whatman GF/C paper using a receptor binding harvester (Brandel Model M-24, Gaithersburg, MD). Filters were removed and counted in 4 ml of scintillation fluid (Formula-989, Packard Instrument Company, Inc., Meriden, CT) at 43% efficiency. Specificity was determined by the inhibition of atropine (10 μM final concentration) in paired samples and calculated as the difference in binding between tissues.
incubated in the presence and absence of atropine. Binding data were calculated relative to protein concentration. CD binding was measured by the method of Ehler and co-workers (1980) with some modifications. Membranes (approximately 250–500 μg of protein per reaction) were incubated with [3H]HClD (4 nM final concentration) in a final volume of 2 ml in the above Tris–salts buffer for 60 min at 37°C. Specificity was demonstrated as with [3H]QNB binding using atropine (10 μM final concentration). The reaction was terminated by vacuum filtration and binding determined by liquid scintillation as described before.

High-affinity choline uptake. [3H]Choline chloride uptake was measured in crude synaptosomes essentially by a modification of the method of Nordberg (1978) as reported previously (Pope et al., 1987). Aliquots (100 μl) of crude synaptosomes (100–200 μg of protein) were preincubated with 380 μl of fresh, oxygenated buffer for 5 min at 37°C followed by the addition of 20 μl of [3H]choline chloride (final concentration of choline adjusted to 0.5 μM with cold choline chloride) and subsequent incubation for another 3 min at 37°C in a final volume of 0.5 ml. The reaction was stopped by addition of 3 ml of ice-cold 1 mM isotonic choline chloride.

The samples were then vacuum filtered and washed with ice-cold 1 mM isotonic choline chloride (3 ml, 3 x) over Whatman GF/C paper using the receptor binding harvester. Filters were collected and counted as described in the receptor binding assays. High-affinity choline uptake activity was determined by incubation of paired samples at 0°C and calculated as the difference in uptake of [3H]choline chloride between paired tissues incubated at 37°C and those incubated at 0°C. Activity was related to protein concentration. The in vitro effect of CPF-oxon and HC-3 on choline uptake was measured by adding inhibitors (1 x 10^{-7} to 3 x 10^{-4} M final concentration) just prior to the 5-min preincubation period and continuing the uptake assay as before.

Binding to [3H]hemicholinium-3. Binding to the specific choline transporter by [3H]HC-3 was performed essentially by the method of Sandberg and Coyle (1985). Washed membranes (100–200 μg of protein) were incubated with [3H]HC-3 (20 nM final concentration) in 50 mM glycyl-glycine buffer, pH 7.8, for 45 min at 25°C in a final volume of 200 μl. Subsequent to incubation, tissues were filtered under reduced pressure and washed with ice-cold glycyl-glycine-glycine buffer (3 ml, 3 x) as described above. Specific binding was measured by the inclusions of cold HC-3 (10 μM final concentration) in paired tissues and calculated as the difference in binding between samples incubated in the presence and absence of cold HC-3. For kinetic analyses, membranes from each treatment group (n = 5/treatment) were incubated with varying concentrations (1.25 to 40 nM final concentration) of [3H]HC-3 and binding data were plotted and analyzed for kinetic parameters (Bmax and Kd) using Rosenthal plots (Tallarida and Murray, 1981). All binding data were related to protein content.

Data analysis. Statistical analyses were performed using the SAS package (SAS, 1988) and the SigmaStat program (Jandel) for personal computers. Parametric neurochemical data were tested for significance by Student’s t test or ANOVA using the SAS General Linear Model procedure followed by Duncan’s test. Median values for SLUD signs or tremors were tested for significance by the Mann Whitney U test, whereas differences in body weight were tested for significance by repeated measures analysis of variance. A probability level of 0.05 was considered significant. Inhibitory potency (IC50) values were calculated using the GraphPad Prism software nonlinear regression (fit) program (Motulsky et al., 1994–1995).

RESULTS

Acute Toxicity

Exposure to a high, subcutaneous dose of CPF (280 mg/kg) caused a significant decrease in body weight in adult female rats from 2 to 7 days after treatment, with maximal reduction (12%) being noted at 3–4 days postexposure (Fig. 1A). Similar to our previous results in male rats (Pope et al., 1991, 1992; Chaudhuri et al., 1993), the high dose of CPF caused relatively minor signs of overt acute toxicity in female rats expressed as either SLUD signs (Fig. 1B) or involuntary movements (tremors) (Fig. 1C). The maximal degrees of SLUD signs (median score, 1.5; p > 0.1) were evident at 3–4 days posttreatment, whereas the highest degrees of involuntary movements (median score, 3.5; p > 0.1) occurred at Days 5–6. None (0/84) of the rats exposed to CPF (280 mg/kg, sc) in these studies showed extensive signs of toxicity, became moribund, or died during the experiment.

AChE Inhibition

Figure 2 shows the degrees of AChE inhibition in frontal cortex 1, 2, or 7 days following CPF exposure. Cortical AChE activity was inhibited >90% at all three time points with maximal inhibition (93%) at 7 days after treatment.
CHOLINE UPTAKE AND CHLORPYRIFOS EXPOSURE

FIG. 2. Effect of CPF (280 mg/kg, sc) on cortical AChE activity. Adult female rats were exposed to CPF and then sacrificed at 1, 2, or 7 days after treatment for measurement of AChE activity in frontal cortex. Data represent percentages (means ± SE, n = 10–12/group/time point) of contemporaneous control values. Combined control value (mean ± SE) for cortical AChE activity was 49.8 ± 2.0 nmol acetylcholine hydrolyzed/min/mg protein (range of means from different time points, 42.0–56.4). Asterisks indicate significant differences (p < 0.05) between treated and control values. Where error bars are unclear, the variability is exceedingly low.

Effects of CPF Exposure on Muscarinic Receptor Binding

Total muscarinic receptor ([3H]QNB) binding and binding to the m2-selective agonist CD in frontal cortex were measured 1, 2, or 7 days after exposure. As we have noted previously in adult male rats (Chaudhuri et al., 1993), a single high dose (280 mg/kg, sc) of CPF caused significant reductions (10–34%) in total muscarinic receptor binding in cortex at all three time points in adult female rats (Fig. 3A). Maximal reduction (34%) occurred at 7 days after treatment, which was significantly different from the other two time points. Binding to [3H]CD, however, was significantly increased (15–23%) by CPF at all three time points with no significant difference among those time points (Fig. 3B).

In Vivo and In Vitro Effects of CPF or CPF-Oxon on High-Affinity Choline Uptake

Crude synaptosomal HACU activity in frontal cortex was measured 1, 2, or 7 days after CPF exposure (Fig. 4A). Cortical HACU was significantly depressed (20%) 1 day following CPF exposure but recovered to control values by 2 days after treatment. Figure 4B shows the comparative in vitro effects of CPF-oxon (the active metabolite of CPF) and the prototype inhibitor HC-3 on HACU. The inhibitory potency (IC50) of HC-3 was 17 ± 1 μM while the IC50 for CPF-oxon appeared greater than 200 μM.

[3H]Hemicholinium-3 Binding

Binding to [3H]HC-3 was reduced in frontal cortex 1 day after CPF (70, 140, or 280 mg/kg, sc) in a dose-related manner which roughly paralleled the degree of AChE inhibition (Fig. 5A). Kinetic analyses of [3H]HC-3 binding following the high dose (280 mg/kg) revealed a significant decrease in density (Bmax: control, 187 ± 18 fmol/mg protein; CPF, 104 ± 12 fmol/mg protein) with no apparent change in the binding affinity (Kd: control, 25 ± 3 nM; CPF, 19 ± 3 nM) relative to controls (Fig. 5B).

DISCUSSION

Similar to other OP insecticides, CPF exerts acute toxicity primarily through AChE inhibition in both the central and the peripheral nervous systems (Ecobichon, 1991). We previously observed, however, that high subcutaneous doses of CPF in adult male rats (associated with >90% AChE inhibition) produced few signs of overt toxicity (Pope et al., 1992; Chaudhuri et al., 1993). In the present study, adult female rats treated with a high single dose of CPF (280 mg/kg, sc) also exhibited relatively minor degrees of overt toxicity (Fig. 1), even with extensive (>90%) and persistent (up to 7 days after exposure) inhibition of cortical AChE activity (Fig. 2). While this study did not provide a direct comparison, in contrast to reported sex-related differences in response to intraperitoneal CPF exposure (Sultatos, 1991), it appears that female rats are not much different relative to
male rats in their response to high-dose subcutaneous CPF challenge.

Extensive and persistent AChE inhibition is often associated with compensatory changes in cholinergic receptors which are generally thought to participate in the development of tolerance to anticholinesterase agents (for a review, see Russell and Overstreet, 1987). When examining the effects of CPF treatment on total muscarinic receptors (using the nonselective antagonist QNB), we observed extensive down-regulation of these binding sites (Fig. 3A). In contrast, binding to the m2-selective agonist CD was significantly increased by CPF exposure (Fig. 3B). These results agree with our previous findings in adult male rats (Pope et al., 1992; Chaudhuri et al., 1993) and provide further evidence that CPF can selectively alter subpopulations of muscarinic receptors.

It has been reported that the active, oxidative metabolite of CPF (i.e., CPF-oxon) binds with high affinity to the subset of muscarinic receptors labeled with \(^{1}H\)CD (Huff et al., 1994). The mode of action of CPF may therefore depend on direct interactions with both AChE and cholinergic receptors. Those cholinergic receptors which bind with high affinity to \(^{1}H\)CD appear to be concentrated in presynaptic cholinergic terminals (Watson et al., 1986) and have been proposed to act as autoreceptors regulating acetylcholine release (Pope et al., 1995). It has also been reported that muscarinic autoreceptors may be coupled to HACU, with antagonists and agonists increasing and decreasing uptake, respectively (Breer and Knipper, 1990). The increased density and possibly enhanced function of m2 muscarinic autoreceptors following CPF exposure could therefore lead to a decrease in HACU/acetylcholine synthesis and a reduction in the amount of acetylcholine released upon stimulation. We propose that direct interaction with CPF-oxon and/or compensatory alterations in these receptors modify acetylcholine synthesis and release and that these responses mitigate the toxicity of extensive AChE inhibition following high-dose CPF exposure.

High-affinity choline uptake, the rate-limiting step in ace-
tylcholine synthesis, is specific for presynaptic cholinergic neurons (Kuhar and Murrin, 1978). Several reports have shown that inhibitors of HACU/acytylcholine synthesis (e.g., acetylsecocomicholinium, N-allyl-3-quinuclidinol, and HCl-3 congeners) can reduce the toxicity of OP AChE inhibitors (Buccafusco and Arostan, 1986, 1987; Sterling et al., 1988; Cannon et al., 1990). In the present study, HACU was reduced at 1 day but similar to the control at 2 and 7 days following CPF exposure (280 mg/kg, sc), even though AChE activity was inhibited >90% at all three time points. Other OPs such as DFP, soman, sarin, and DDVP have also been reported to reduce HACU early (20 min to 4 hr) after exposure (Lim et al., 1987; Whalley and Shih, 1989; Kobayashi et al., 1986), suggesting that HACU may be an additional "target" for some OP compounds. In order to determine whether HACU is affected directly by CPF, the in vitro effect of CPF-oxon (the active metabolite of CPF) on HACU was examined in comparison to HC-3, a well-known HACU inhibitor. CPF-oxon appeared to be a weak inhibitor of HACU (IC50 > 200 μM) relative to HC-3 (IC50 = 17 μM). The results suggest that CPF reduces HACU early after exposure but not through direct interaction with the choline transporter. A similar conclusion was also reached by Whalley and Shih (1989) regarding interactions between soman and sarin and the high-affinity choline transport system.

[^H]Hemicholinium-3 is a specific, reversible ligand for the high-affinity choline transporter. It has been reported that the degree of binding of[^H]HC-3 to the choline transporter is correlated with HACU in cholinergic neurons and that[^H]HC-3 binding can provide a simple index of choline uptake (Sandberg and Coyle, 1985; Swann and Hewitt, 1988). One advantage of[^H]HC-3 binding is that it does not require intact, functioning nerve endings as would be required for the measurement of choline uptake. Binding to[^H]HC-3 may also be a more sensitive indicator for detecting changes in the high-affinity choline transport system than[^H]choline uptake into synaptosomes (Lowenstein and Coyle, 1986). We therefore used[^H]HC-3 binding to characterize further the early change in choline transport following CPF exposure. A dose—response study 1 day after CPF revealed a dose-related decrease in[^H]HC-3 binding (Fig. 5A). While binding to[^H]HC-3 was reduced at higher dosages (140 and 280 mg/kg) causing 82—87% inhibition of cortical AChE activity, no significant reduction in binding was noted with the lower dosage of CPF (70 mg/kg), resulting in lesser but still extensive (75%) AChE inhibition. Additionally, Whalley and Shih (1989) reported that sarin could reduce cortical choline uptake within 1 hr after treatment but in a manner apparently independent of AChE inhibition. Thus, AChE inhibition may not be directly coupled to alterations in HACU following OP exposure.

Kinetic analysis of[^H]HC-3 binding 1 day after CPF (280 mg/kg, sc) indicated a significant reduction in binding density (Bmax) with no apparent change in the binding affinity (Kd) (Fig. 5B). Similar findings (i.e., changes in Bmax but not Kd) have been reported by Lowenstein and Coyle (1986) with other cholinergic treatments (i.e., atropine and oxotremorine) in rats. Taken together, these results suggest that CPF can reduce cortical HACU via an indirect mechanism through a reduction in the density of transporter molecules on presynaptic terminals, possibly independent of AChE inhibition.

In summary, this study has provided further evidence that an acute, high dose of CPF (280 mg/kg, sc) can be associated with extensive and persistent AChE inhibition but minimal signs of toxicity. An early reduction in HACU/acytylcholine synthesis may lessen the consequences of CPF-induced AChE inhibition. It is apparent, however, that multiple presynaptic and postsynaptic cholinergic processes can be altered by CPF and together these changes may contribute to the resistance to overt toxicity and to the development of tolerance to this OP.

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