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Novel Effects of Memantine in Antagonizing Acute Aldicarb Toxicity: Mechanistic and Applied Considerations

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ABSTRACT

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Administration of a single sublethal dose of aldicarb (0.4 mg/kg, i.p.) to male Sprague-Dawley rats produced the onset of hypercholinergic signs within 3–5 min. With increasing intensity the most severe signs, predominantly involving the peripheral nervous system (muscle fasciculations and convulsions), were evident within 15–30 min and lasted for about 90 min. Rats dosed with 0.1 mg/kg, i.p. did not develop intoxication while those dosed with 0.2 mg/kg, i.p. exhibited signs of moderate toxicity. A dosage of 0.6 mg/kg was found to be the minimal lethal dose (MLD). The inhibition of acetylcholinesterase (AChE) and carboxylesterase (CarbE) was dose-dependent. Significant ($P<0.01$) inhibition in the activities of AChE and CarbE occurred as early as within 15 min, and the maximal effect within 30 min. Time course of CarbE activity revealed marked inhibition in different neuronal and nonneuronal tissues, suggesting tremendous nonspecific binding of aldicarb. Prophylaxis with memantine HCl (18 mg/kg, i.p.) and atropine sulfate (16 mg/kg, i.p.), 30 min and 15 min, respectively, prior to aldicarb (0.4 mg/kg), provided complete protection. Therapeutic administration of these two antidotes in combination also completely reversed the clinical signs of intoxication. It is suggested that memantine antagonized the aldicarb acute toxicity by protection/reactivation of activities of AChE and CarbE and reversible blockade of hyperneuromuscular transmission, in addition to muscarinic ACh receptor blockade by atropine.

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Key words: carbamate toxicity, acetylcholinesterase, carboxylesterase, antidotal treatment

INTRODUCTION

Carbamate insecticides are considered safer than organophosphate (OP) insecticides because they produce poisoning by virtue of reversible inhibition of acetylcholinesterase (AChE). Aldicarb, which is not only one of the most toxic carbamates, but also more toxic than several OP insecticides, has recently been encountered in several accidental poisonings in animals [Spierenburg et al., 1985; Dorman et al., 1990; Kerr et al., 1991] as well as in humans [Goes et al., 1980; Lee and Ransdell, 1984; Parks et al., 1987; Goldman et al., 1990].

Although the mechanism of toxic action of aldicarb is reported to be similar to other carbamate insecticides, i.e., reversible AChE inhibition at the synapses and neuromuscular junctions [Casida, 1963; O'Brien, 1967; Cambon et al., 1979] leading into accumulation of acetylcholine, the toxic manifestations differ very widely. For example, carbofuran, propoxur, and carbaryl produced toxic signs involving central as well as peripheral nervous systems [Gupta and Kadel, 1989a,b, 1990b], whereas, aldicarb in preliminary studies exhibited signs predominantly of the peripheral nervous system.

Unlike AChE, carboxylesterases (CarbEs), which are not known to have any physiological role per se, constitute a large pool for nonspecific binding (false targets) of carbamates and OPs, and provided a partial protection against these anticholinesterase insecticides [Gupta and Kadel, 1989a,b, 1990a]. These findings were in contrast to the earlier reports [Myers and Mendel, 1949; Earl et al., 1953; Takahashi et al., 1987] suggesting that some carbamate compounds (physostigmine, pyridostigmine, and 2-Sec-butylphenyl methylcarbamate) do not inhibit CarbEs.

Antidotal treatment with atropine sulfate is recommended in carbamate poisoning, as it is in organophosphate poisoning, while therapy with oximes such as pralidoxime is mostly unsuccessful and in some cases contraindicated [Hayes, 1982; Murphy, 1985]. Atropine sulfate antagonizes the muscarinic hypersecretory aspects of carbamate toxicity (salivation, lacrimation, tracheobronchial secretion, diarrhea, etc.); however, the nicotinic receptor-associated effects (muscle tremors and fasciculations, convulsions, etc.) always remain unprotected. Unfortunately, no single therapeutic agent is yet available to counteract both muscarinic and nicotinic effects. Recently, a combined treatment with memantine HCl (MEM) and atropine sulfate (ATS) was found to be one of the most effective antidotes against anti-AChE poisoning due to carbofuran and methyl parathion [Gupta and Kadel, 1989b, 1990b].

The present investigation was therefore undertaken with two specific objectives: (1) to study the characteristic profile of clinical and biochemical changes by acute aldicarb toxicity and (2) to evaluate the prophylactic and therapeutic potential of MEM and ATS as antidotes. Perhaps future investigations on higher species, such as dogs and non-human primates, will further validate our present findings on the use of MEM as a novel antidote in anticholinesterase insecticides poisoning.

MATERIALS AND METHODS

Chemicals

Aldicarb (2-Methyl-2-[methylthio]propionaldehyde]-0-[methylcarbamoyl] oxime), technical grade (98%), in a powder form was purchased from Chem Service Inc. (West Chester, PA). Atropine sulfate (ATS), acetylthiocholine iodide, tetraisopropylpyrophosphoramide (iso-OMPA), tributyrin (C4:0), and hydroxylamine HCl were purchased from Sigma Chemical Co. (St. Louis, MO). Memantine HCl (1,3-dimethyl-5-aminoadamantane HCl, MEM) was received as a gift from Merz & Co. (Frankfurt, FRG). No further purification or verification of their purity was carried out in our laboratory. All other chemicals of highest purity were purchased from Fisher Scientific (Fair Lawn, NJ). The solutions of aldicarb in

TABLE 1. Dosage Schedule of Antidotal Treatment (MEM and/or ATS) in Rats Acutely Intoxicated With Aldicarb (0.4 mg/kg, i.p.)*

Treatment	Group	Toxicant/antidote(s)	Schedule of antidotal treatment
Control	I	DMSO	None
Intoxication	II	Aldicarb	None
Prophylactice treatment	III	MEM + Aldicarb	MEM 30 min before Aldicarb
	IV	ATS + Aldicarb	ATS 15 min before Aldicarb
	V	MEM + ATS + Aldicarb	MEM 30 min and ATS 15 min before Aldicarb
Therapeutic treatment	VI	Aldicarb + MEM	MEM 5–7 min after Aldicarb
	VII	Aldicarb + ATS	ATS 5–7 min after Aldicarb
	VIII	Aldicarb + MEM + ATS	MEM and ATS 5–7 min after Aldicarb

*Drugs and dosages: memantine HCl (MEM, 18 mg/kg, i.p.); atropine sulfate (ATS, 16 mg/kg, i.p.). Sacrifice time: rats were sacrificed 30 min after aldicarb administration.

dimethyl sulfoxide (DMSO) and ATS and MEM in normal saline (0.9% NaCl) were freshly prepared, just prior to injections, and the remainders were destroyed after deactivation with 2.5 N NaOH.

Animals

Male Sprague-Dawley rats weighing between 180 and 200 g were purchased from S.D. Sasco (St. Louis, MO) and were acclimatized to our standard laboratory conditions for 7–10 days before being used in these experiments. The animals were kept under a light/dark schedule of 12 hr/12 hr at 21±1°C and 50 ±10% relative humidity. They were allowed free access to food (Rodent Laboratory Chow, Purina Mills, Inc., St. Louis, MO) and tap water.

Experimental Protocol

This investigation was based on two sets of experiments.

Acute toxicity of aldicarb. Rats, five in each group, were dosed with 0.1, 0.2, 0.4, or 0.6 mg aldicarb/kg body wt, i.p. Rats were closely observed for obvious toxic signs. Preliminary findings revealed: 0.1 mg/kg, no obvious toxicity; 0.2 mg/kg, moderate toxicity; 0.4 mg/kg, severe toxicity; and 0.6 mg/kg, lethality (MLD). Following a dosage of 0.4 mg aldicarb/kg, the onset of hypercholinergic signs was evident within 3–5 min, maximal severity occurred within 30 min, and recovery started past 90 min. For biochemical analyses, rats were sacrificed at 0.25, 0.5, 1, 3, 6, and 24 hr after aldicarb injection (0.4 mg/kg, i.p.). The maximal inhibition of AChE and carboxylesterase (CarbE) activities was noted at 30 min, the time when severity of intoxication was also at the peak. Based on these findings, rats receiving a single dosage of 0.1 mg/kg or 0.2 mg/kg body wt were also sacrificed at 30 min for enzymatic assays.

Antidotal treatment. Details of the dosage schedule for antidotal treatment in rats acutely intoxicated with aldicarb (0.4 mg/kg, i.p.) are presented in Table 1. The rationale for the selection of MEM and ATS doses is discussed elsewhere [Patterson et al., 1988; Gupta and Kadel, 1989b, 1990b]. Briefly, MEM with a 5, 10, 15, or 20 mg/kg, i.p. dose did not produce any untoward effects in control (untreated) rats. With higher doses (30 or 40 mg/kg, i.p.), however, it evoked hyperexcitability with characteristic of dopaminomimetic action. A low dose of MEM (10 mg/kg, i.p.) provided only partial protection against aldicarb acute toxicosis. Therefore, a dose of 18 mg/kg MEM, reported to be effective as an anticonvulsant dose

against parkinsonism and other related disorders, was employed. MEM at this dose provided full protection or reversal of nicotinic-induced effects of aldicarb. Muscarinic effects remained unprotected, however. On the other hand, ATS with a standard recommended dose (16 mg/kg, i.p.) in rats provided full protection or reversal of muscarinic-induced effects of aldicarb, but no protection of nicotinic effects was seen. A dose of 10 mg atropine/kg, i.p., afforded only partial protection of muscarinic effects. Thus, a combination of MEM (18 mg/kg) with ATS (16 mg/kg) was used because it provided the optimum prophylactic or therapeutic effects against acute aldicarb toxicosis.

The maximal severity of obvious toxic signs (muscarinic: salivation and lacrimation; nicotinic: tremors, fasciculations, and convulsions) following an acute dose of aldicarb (0.4 mg/kg, i.p.) was attained within 30 min. The degree of protection or reversal of these signs by MEM or ATS alone or in combination was graded on the sale of − to + + + + . A − was considered as not effective (no protection or no reversal of any signs), whereas + + + + was considered as highly effective (complete protection or reversal of all toxic signs). +, + +, and + + +, slightly effective, moderately effective, and very effective, respectively, were graded in between − (no protection) and + + + + (complete protection). Rats were sacrificed 30 min after aldicarb administration.

Rat Sacrifice and Tissue Collection

At predetermined times (as mentioned before), rats were sacrificed by decapitation, and tissues (brain, heart, liver, and hemidiaphragm) were excised quickly. Brains were further dissected into four discrete regions (cortex, stem, striatum, and hippocampus). Serum samples were harvested following centrifugation (3,000 rpm for 30 min) of clotted blood within 1 hr of sacrifice. The samples were stored at −70°C and were processed for biochemical analyses within 24 hr to avoid any storage changes.

Tissue Preparation

The tissues were minced with scissors and homogenized (Brinkman Polytron homogenizer, setting 5) in 20 volumes of ice-cold 50 mM phosphate buffer (pH 8.0) for 30 sec. Hemidiaphragm and heart, being tough tissues, were also sonicated for 10 sec by using a Biosonik equipped with a microprobe (probe setting 40).

Assay of Acetylcholinesterase Activity (AChE, EC 3.1.1.7.)

An aliquot of 100–200 μl homogenate (5–10 mg tissue) was preincubated for 30 min with iso-OMPA (1×10^{-5}M), a selective inhibitor of butyrylcholinesterase (BuChE), and then AChE activity was assayed according to the method of Hestrin [1950] with some necessary modifications [Gupta and Dettbarn, 1987; Gupta and Kadel, 1989a] by using acetylthiocholine iodide as the substrate. The enzyme activity was calculated as μmole substrate hydrolyzed/g tissue/hr and expressed in terms of percentage remaining activity compared to controls (100%).

Assay of Carboxylesterase Activity (CarbE, Tributyrinase; EC 3.1.1.1.)

The activity of CarbE was assayed in serum and tissues according to the method of Hestrin [1950] with some necessary modifications [Gupta et al., 1985; Gupta and Dettbarn, 1987], using tributyrin as the substrate. The enzyme activity was calculated as μmole substrate hydrolyzed/g tissue/hr and expressed in terms of percentage remaining activity compared to controls (100%).

Statistical Analyses

Data were statistically evaluated by using analysis of variance (ANOVA) coupled with Duncan's new multiple range test [Steel and Torrie, 1980].

TABLE 2. Antidotal Effectiveness of MEM (18 mg/kg, i.p.) and/or ATS (16 mg/kg, i.p.) in Rats Acutely Intoxicated With Aldicarb (0.4 mg/kg, i.p.)*

		Clinical signs				
		Muscarinic		Nicotinic		
	Treatment	Salivation	Lacrimation	Tremors	Fasciculations	Convulsions
Control	DMSO	None	None	None	None	None
	MEM	None	None	None	None	None
	ATS	None	None	None	None	None
	MEM and/ ATS	None	None	None	None	None
Intoxication	Aldicarb	Excessive	Excessive	Severe	Severe	Severe
		Antidotal effects of MEM and/or ATS				
Prophylaxis	MEM + Aldicarb	+	+	+ + + +	+ + + +	+ + + +
	ATS + Aldicarb	+ + + +	+ + + +	+	−	−
	MEM + ATS + Aldicarb	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
Therapy	Aldicarb + MEM	+	+	+ + + +	+ + + +	+ + + +
	Aldicarb + ATS	+ + + +	+ + + +	+	−	−
	Aldicarb + MEM +ATS	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +

*Antidotal effectiveness graded as: −, not effective; +, slightly effective; + +, moderately effective; + + +, very effective; + + + +, highly effective.

RESULTS

The observations on overt toxic signs following varying doses of aldicarb revealed: 0.1 mg/kg, no obvious toxic signs; 0.2 mg/kg, moderately toxic signs (hypersalivation, lacrimation, tremors and intermittent mild fasciculations); and 0.4 mg/kg, severe intoxication including strong muscle fasciculations and convulsions. A dosage of 0.6 mg aldicarb/kg caused lethality within 10–15 min (data not shown). Following a single acute dosage of 0.2 or 0.4 mg aldicarb/kg, the onset of toxic signs, such as chewing movements and hindleg muscle tremors, was evident within 5 min. With increasing propensity, the maximal severity was evident within 15–30 min and lasted for about 90 min. Thereafter, a rapid recovery was evident, as by the end of 2 hr rats were free from toxic signs. After 3 hr rats were eating, drinking, and grooming, and were found apparently normal.

Antidotal pretreatment with MEM (18 mg/kg) and ATS (16 mg/kg), 30 min and 15 min, respectively, prior to aldicarb (0.4 mg/kg) administration provided full protection against anticipated aldicarb intoxication (Table 2). This combined antidotal treatment, when given within 5–7 min after aldicarb injection as a therapeutic measure, attenuated all the toxic signs within 15–20 min. At no time did animals receiving pretreatment or treatment with these antidotes exhibit recurrence of aldicarb intoxication. Therefore, at no time was these any necessity to repeat antidotal therapy. Pretreatment with MEM alone provided full protection against nicotinic-induced effects while ATS alone provided protection against muscarinic effects. Therapeutic administration of MEM or ATS alone antagonized the induced nicotinic and muscarinic effects correspondingly (Table 2). Pretreatment with MEM in combination with ATS also provided full protection against rats challenged with lethal dose (0.6 mg/kg) of aldicarb (data not shown). Neither MEM and ATS, either alone or in combination, in aldicarb-treated or non-aldicarb-treated rats, produced any observable side effects.

Following a single sublethal acute dosage of aldicarb (0.4 mg/kg) the time courses of

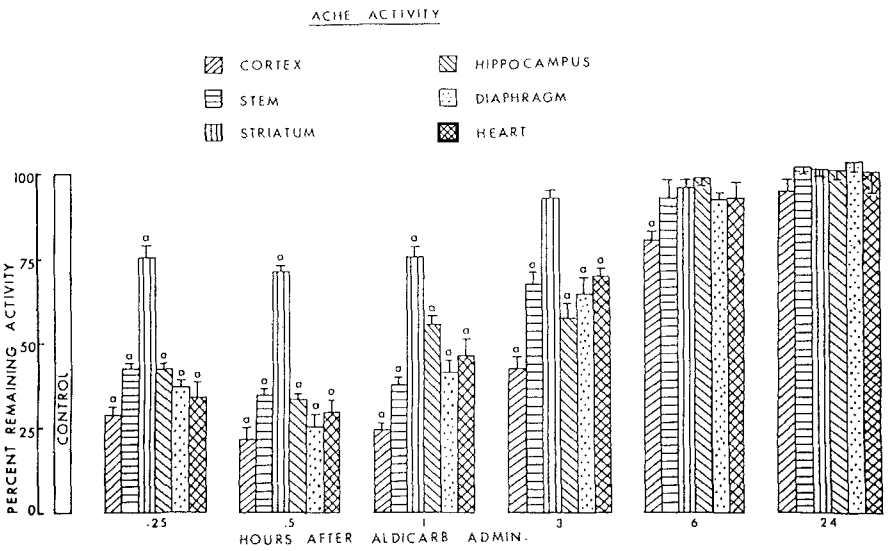

Fig. 1. Time course of effects of an acute sublethal dose of aldicarb (0.4 mg/kg, i.p.) on acetylcholinesterase (μmole acetylthiocholine iodide hydrolyzed/g/hr) activity in the brain regions, diaphragm, and heart. Values are means ± SE presented as percent activity of controls (100%). a = Significant difference between controls and aldicarb treated rats ($P<0.01$).

AChE and CarbE revealed maximal inhibition of their activities within 15–30 min (Figs. 1, 2). Among brain regions, AChE activity was maximally depressed in cortex and least in striatum (Fig. 1), whereas CarbE was maximally depressed in stem and least in cortex (Fig. 2). At 30 min following 0.1 and 0.2 mg/kg doses of aldicarb, the degree of inhibition of AChE and CarbE was dose-dependent, although not linear (Tables 3, 4). By 1 hr, AChE activity already showed moderate recovery (hippocampus, diaphragm, and heart), while CarbE activity was still maximally inhibited in all tissues (except liver and brain stem) and serum. All the brain regions (except cortex), diaphragm, and heart revealed AChE activity in the normal range at 6 hr. At this time brain regions, diaphragm, and heart also showed CarbE activity in the normal range while liver and serum still showed significant ($P < 0.01$) inhibition. At 24 hr post-administration all the tissues regained the activities of both enzymes to normal levels. Heart, in spite of having substantially higher CarbE activity, indicated the least inhibition with either dose of aldicarb. MEM alone or in combination with ATS had no effect on AChE or CarbE activity (data not shown), but significantly protected against and/or attenuated the inhibitory effect of aldicarb (Figs. 3, 4).

DISCUSSION

Toxicity

Rats poisoned with an acute sublethal dosage of aldicarb (0.4 mg/kg, i.p.) exhibited toxic signs predominantly of peripheral origin, while no significant difference was seen between the muscle and the brain regions (except striatum) in terms of the degree of AChE inhibition (Fig. 1). Such predominant peripheral intoxication was found with diisopropylphosphorofluoridate (DFP), an OP compound, despite the fact that the inhibition of AChE was slightly greater in the brain than in the muscles [Gupta et al., 1985, 1986]. In contrast to

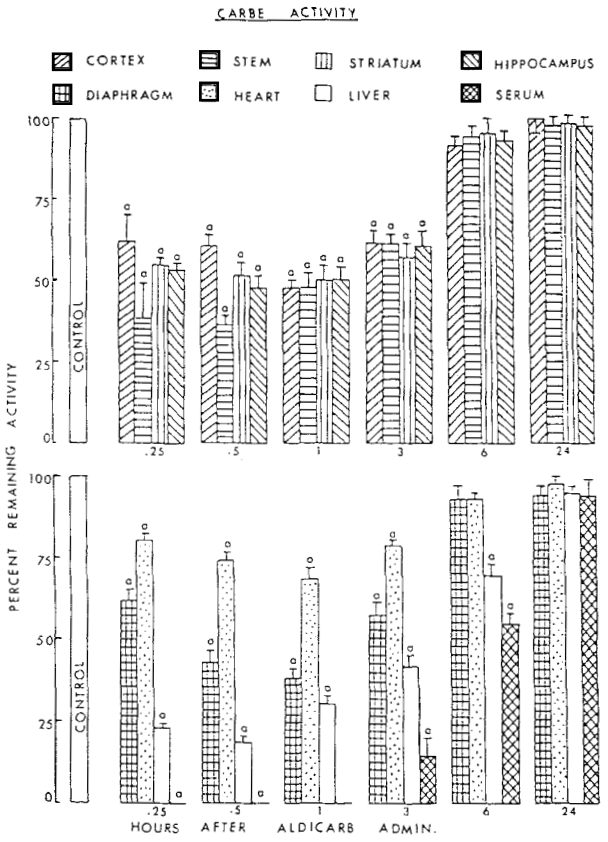

Fig. 2. Time course of effects of an acute sublethal dose of aldicarb (0.4 mg/kg, i.p.) on carboxylesterase (μmole tributyrin hydrolyzed/g/hr) activity in the brain regions, diaphragm, heart, liver, and serum. Values are means ± SE presented as percent activity of controls (100%). a = Significant difference between controls and aldicarb-treated rats ($P<0.01$).

aldicarb, other carbamate insecticides (carbofuran, propoxur, and carbaryl) produced intoxication of central as well as peripheral origins [Gupta and Kadel, 1989a, 1990a].

With lower doses of aldicarb, AChE activity was more inhibited in the muscle than in the brain regions, which could be due to easy access of aldicarb to the muscles, following an intraperitoneal route of administration. The greater inhibition of CarbE in serum and liver (100% and 81.5%, respectively) convincingly suggested substantive nonspecific binding of aldicarb, to serine-containing non-AChE enzyme(s), primarily in the peripheral system, thereby reducing free concentration of aldicarb available to the brain. In other words, CarbE binding may subserve the protection against aldicarb toxicity, particularly when exposure is at low levels. The finding of CarbEs inhibition by acute aldicarb intoxication was in agreement with our recent reports [Gupta and Kadel, 1989a, 1990a] on other N-methylcarbamates (carbofuran and propoxur), while in contrast to reports of other investigators [Myers and Mendel, 1949; Earl et al., 1953; Takahashi et al., 1987] who reported that some carbamates such as physostigmine, pyridostigmine, and 2-sec-butylphenyl methylcarbamate (BPMC) do not inhibit CarbEs. At this point the question remains unanswered as to why the heart CarbE was least affected among all the tissues tested, despite the heart's high activity of CarbE and greater access to aldicarb.

TABLE 3. Acetylcholinesterase Activity in Tissues of Rats, at 30 min (Time of Maximal Severity), Following an Acute Injection of Aldicarb (0.1, 0.2, or 0.4 mg/kg, i.p.)*

	Acetylcholinesterase (% of control) activity (means ±SE; n = 5)			
Tissues	DMSO (control)	0.1 mg/kg	0.2 mg/kg	0.4 mg/kg
Brain regions				
Cortex	100 (266.4 ± 8.8)	73.4 ± 0.9[a]	43.2 ± 1.1[a]	21.6 ± 3.6[a]
Stem	100 (504.0 ± 10.7)	71.9 ± 1.9[a]	52.9 ± 1.9[a]	34.3 ± 1.8[a]
Striatum	100 (1596.0 ± 30.6)	94.9 ± 2.4	83.2 ± 1.7	71.6 ± 1.5[a]
Hippocampus	100 (357.6 ± 11.0)	84.6 ± 1.9	52.3 ± 2.3[a]	33.6 ± 1.5[a]
Muscles				
Diaphragm	100 (99.2 ± 2.0)	66.1 ± 4.7[a]	27.4 ± 3.0[a]	25.4 ± 3.2[a]
Heart	100 (44.4 ± 2.7)	62.3 ± 6.4[a]	31.1 ± 3.0[a]	29.2 ± 3.5[a]

*Rats were sacrificed 30 min after aldicarb administration. Values of acetylcholinesterase activity, determined as μmole acetylthiocholine iodide (ACh) hydrolyzed/g tissue/hr, are presented as percent activity of controls (100%). Values in parentheses, DMSO-treated control, are μmole ACh hydrolyzed/g/hr.
[a] = Significant difference between controls and aldicarb-treated rats ($P<0.01$).

The present results also revealed an important finding; i.e., AChE activity was least inhibited in the striatum (Fig. 1, Table 3) by aldicarb, despite the fact that the striatum has the highest AChE activity among all the major discrete brain regions. Although the striatum had the lowest inhibition of AChE on a percent basis, it normally had three to five times higher AChE activity and thus, in absolute terms, might have equal or greater inhibition of AChE activity. Brain regions with much lower AChE activity showed the highest percentage inhibition mainly because they contained lower numbers of binding sites. Similar observations were also noted following subacute aldicarb administration [Gupta and Kadel, 1991]. To our knowledge this finding is in accordance only with an OP nerve agent VX (S-(2-diisopropylaminoethyl) 0-ethyl methylphosphonothioate) while in contrast to most carbamate and OP compounds [Gupta et al., 1986, 1987, 1991; Gupta and Kadel, 1989a, 1990a,b]. It can be speculated that a proportionally higher percent of remaining (uninhibited) AChE activity in the striatum (> 71%) during peak aldicarb poisoning might act as a protective mechanism and might also be the reason for the weak toxic signs associated with the central nervous system.

Treatment

The oximes are not effective in antagonizing the toxicity of the carbamyl ester inhibitors, and since pralidoxime itself has weak anti-ChE activity, they are contraindicated in the treatment of overdosage with pyridostigmine or physostigmine or poisoning with carbaryl [Hayes, 1982; Murphy, 1985]. Our preliminary findings further confirmed that pretreatment or treatment with pralidoxime did not protect or reverse the acute toxicity of aldicarb or carbofuran. In similar trials, pretreatment or treatment with diazepam rather accentuated the toxicity of these carbamates.

In our recent studies we demonstrated that a combination of two antidotes (MEM and ATS) was far superior to any other antidote(s) against acute toxicity of carbofuran [Gupta and Kadel, 1989b]. These antidotes, in combination, provided complete protection against acute

TABLE 4. Carboxylesterase Activity in Tissues and Serum of Rats, at 30 min (Time of Maximal Severity), Following an Acute Injection of Aldicarb (0.1, 0.2, or 0.4 mg/kg, i.p.)*

	Carboxylesterase (% remaining) activity (mean±SE; n = 5)			
Tissues	DMSO (control)	0.1 mg/kg	0.2 mg/kg	0.4 mg/kg
Brain regions				
Cortex	100 (68.8 ± 2.6)	74.4 ± 2.8[a]	65.6 ± 3.0[a]	60.3 ± 3.1[a]
Stem	100 (68.8 ± 2.3)	74.4 ± 4.3[a]	62.6 ± 3.4[a]	36.0 ± 2.2[a]
Striatum	100 (71.2 ± 1.5)	69.0 ± 4.8[a]	57.1 ± 4.5[a]	51.7 ± 4.1[a]
Hippocampus	100 (72.0 ± 3.1)	76.7 ± 2.7[a]	54.2 ± 3.1[a]	47.8 ± 3.8[a]
Muscles				
Diaphragm	100 (67.2 ± 2.0)	76.2 ± 4.4[a]	54.8 ± 4.4[a]	42.8 ± 3.5[a]
Heart	100 (92.8 ± 2.9)	83.6 ± 3.2	76.7 ± 1.6[a]	74.1 ± 2.5[a]
Liver	100 (3014 ± 60)	51.3 ± 3.2[a]	38.7 ± 2.0[a]	18.5 ± 1.8[a]
Serum	100 (30.7 ± 2.0)	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]

*Rats were sacrificed 30 min after aldicarb administration. Values of carboxylesterase activity, determined as μmole tributyrin hydrolyzed/g tissue or serum/hr, are presented as percent activity of controls (100%). Values in parentheses, DMSO-treated control, are μmole tributyrin hydrolyzed/g tissue/hr. [a] = Significant difference between controls and aldicarb-treated rats ($P<0.01$).

aldicarb intoxication when given as prophylaxis and also completely reversed the clinical signs when administered as therapeutic measures (Tables 1, 2), although aldicarb was about four times more toxic than carbofuran and also produced a somewhat different toxic syndrome. In addition, this antidotal treatment was found equally effective in rats subchronically intoxicated with aldicarb [Gupta and Kadel, 1991]. MEM itself did not produce any side effects [Miltner, 1982] with the exception of slight excitement or hyperexploratory cage activity [Gupta and Kadel, 1989b], which imparts an obvious advantage over other antidote(s).

The mechanism of action of ATS in partial protection and reversal of anticholinesterase agent poisoning is well established [Hulme et al., 1978; Taylor, 1985]. In brief, ATS competitively blocks the muscarinic receptors and thereby attenuates the hypersecretory activities of accumulated acetylcholine (ACh). The mechanism of action of MEM in antagonizing the anticholinesterase poisoning, however, seems more complex. The present data reveals that MEM ameliorated the acute toxicity of aldicarb mainly by protection and rapid reactivation of AChE (Fig. 3), which consequently might have lowered the accumulated ACh levels. In addition to protection of AChE, MEM also afforded protection of restoration of CarbE (Fig. 4), which might have consequently provided (1) nonspecific binding sites for aldicarb and (2) rapid degradation of aldicarb, thus enhancing bioelimination of aldicarb in a similar fashion to that reported for carbofuran and methyl parathion [Gupta and Kadel, 1989b, 1990b].

The blockade of nicotinic effects by MEM in aldicarb intoxication could be due to blocking of nicotinic receptors through interaction with the ACh receptor-ion channel complex [Masuo et al., 1986], and central muscle relaxation [Grossman and Jurna, 1977], thereby causing reversible neuromuscular blockade. However, MEM without ATS did not provide

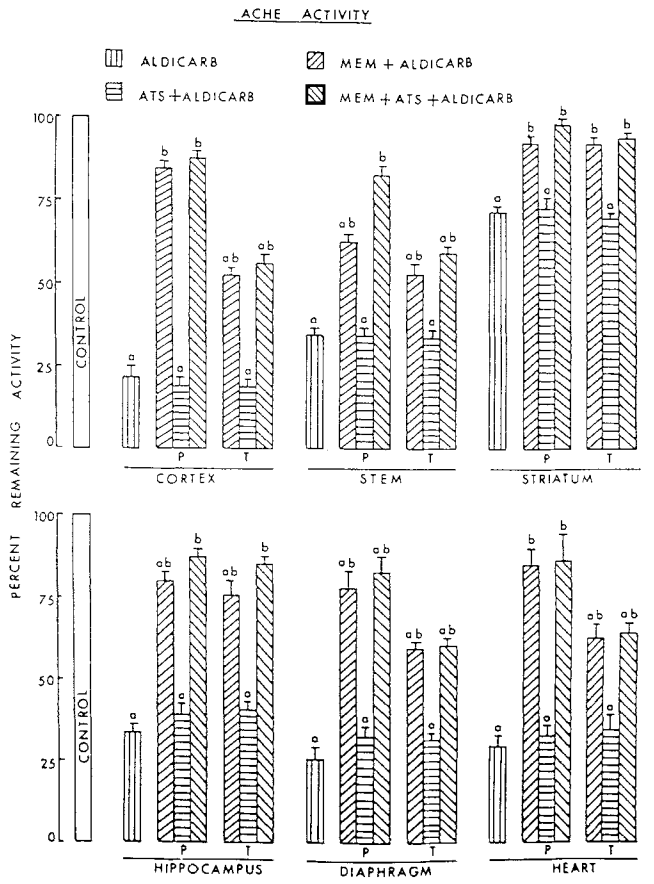

Fig. 3. Effects of MEM (18 mg/kg, i.p.) and/or ATS (16 mg/kg, i.p.), given prophylactically (P) or therapeutically (T), on AChE activity in brain regions, diaphragm, and heart of the rats exposed to aldicarb (0.4 mg/kg, i.p.). Rats were sacrificed 30 min after aldicarb administration. Values are means ± SE presented as percent activity of controls (100%). For details see Figure 1 and Methods. a = Significant difference between controls and treated rats ($P<0.01$). b = Significant difference between aldicarb and aldicarb + antidote (MEM and/or ATS)-treated rats ($P<0.01$).

protection against muscarinic effects of aldicarb (Table 2), which suggested that a sufficient amount of accumulated ACh still remained unhydrolyzed to which muscarinic receptors showed comparatively greater sensitivity. Perhaps MEM lacks a direct effect on muscarinic receptors. Similar observations have also been reported with MEM against carbofuran or methyl parathion [Gupta and Kadel, 1989b, 1990b] and with pyridine 2-aldoxime methiodide (2-PAM) against malathion [Gupta, 1984].

MEM, a well-studied antiparkinson drug, is known to exert multiple actions. In addition to several synaptic and nonsynaptic mechanisms, (1) reduction of permeability of axonal membranes to Na^+ and Ca^{2+}, limiting high-frequency repetitive activation of peripheral nerves [Wesemann and Ekenna, 1982; Wesemann et al., 1983], (2) prevention of neural hyperexcitability [McLean, 1987], and (3) reduction of the reflex excitability of both flexors and extensors in decerebrated cats [Wand et al., 1977] further strengthen our conclusion that MEM exhibits anticonvulsant action against acute aldicarb toxicity.

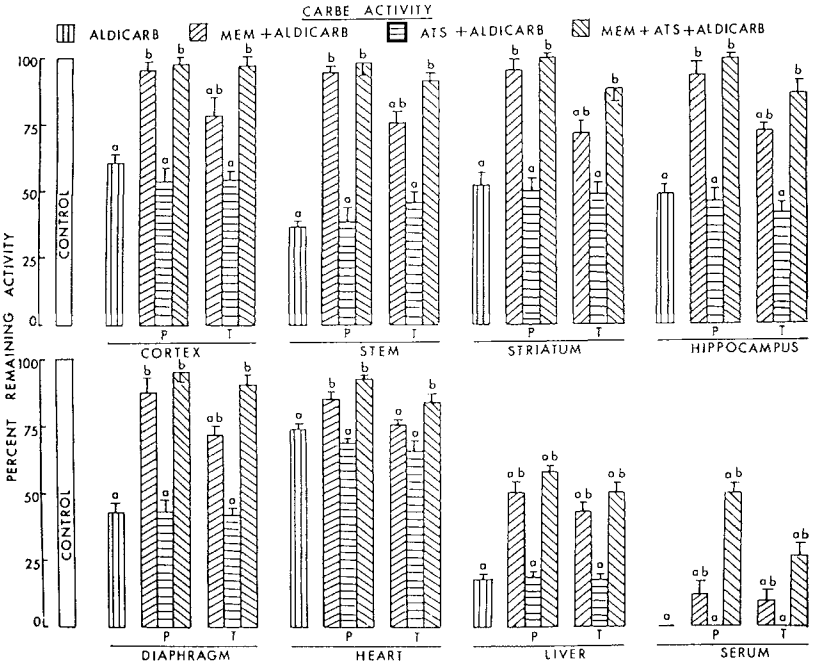

Fig. 4. Effects of MEM (18 mg/kg, i.p.) and/or ATS (16 mg/kg, i.p.), given prophylactically (P) or therapeutically (T), on CarbE activity in brain regions, diaphragm, heart, liver, and serum of the rats exposed to aldicarb (0.4 mg/kg, i.p.). Rats were sacrificed 30 min after aldicarb administration. Values are means ± SE presented as percent activity of controls (100%). For details see Figure 1 and Methods. a = Significant difference between controls and treated rats ($P<0.01$). b = Significant difference between aldicarb and aldicarb + antidote (MEM and/or ATS)-treated rats ($P<0.01$).

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