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Effects of carbaryl, permethrin, 4-nonylphenol, and copper on muscarinic cholinergic receptors in brain of surrogate and listed fish species

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Abstract

We investigated the regulation of the muscarinic cholinergic receptor (MChR) in brain from seven species of fish, two surrogates and five threatened or endangered species exposed to a series of chemicals as a measure of compensatory response among species. Fish were classified as either cold water (rainbow trout-surrogate, apache trout, lahanton trout) or warm water (fathead minnow-surrogate, razorback sucker, bonytail chub, colorado squawfish) and were exposed to chemicals shown to affect cholinergic pathways (carbaryl and permethrin) and two chemicals whose relationships to the cholinergic system is less clear (4-nonylphenol and copper). Downregulation of MChR occurred in all warm water species, except colorado squawfish, and at carbaryl concentrations similar to those causing downregulation observed in rainbow trout. Permethrin exposure resulted in downregulation in fathead minnow and razorback sucker, but the concentrations required for observation of this phenomenon were much greater than observed in cold water species. Copper exposure caused a decrease in brain MChR in rainbow trout and apache trout, whereas 4-nonylphenol exposure resulted in a decrease in brain MChR in all three cold water species. Our results indicate that surrogates are useful in assessing sublethal physiological responses to chemicals with a known mechanism of action such as carbaryl and support use of surrogates for assessing physiological responses to chemicals with diverse, less clear mechanisms of action.

Keywords: Carbaryl; Copper; Downregulation; Fathead minnow; Muscarinic receptors; Nonylphenol; Permethrin; Rainbow trout; Surrogate

1. Introduction

The number of pesticides and other commercial chemicals currently in use coupled with multiple toxic mechanisms of action of these chemicals, often poses a risk to nontarget organisms, including endangered or threatened species (listed). These risks can range from a sublethal-no adverse effect, to a sublethal-adverse effect, to a lethal effect. Since, by definition, the distribution of listed species is limited, one approach to studying potential effects of chemicals on listed species is to utilize surrogate species from the same or a related taxonomic family. Surrogates allow a more thorough study of the sublethal and lethal effects of chemicals without compromising a threatened population. Since even closely related surrogates may not accurately represent threatened populations, a comparison of a physiological effect, such as adaptation, to a sublethal chemical concentration by listed species and representative surrogates would be beneficial in evaluating the utility of using surrogates.

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The mechanism of action of several pesticides is inhibition of brain acetylcholinesterase (AChE). These pesticides include organophosphates, carbamates, and some organochlorines [5,18,31,32]. In addition, there are reports indicating pyrethroids' effect on the cholinergic system [1,13,30], although pyrethroids appear to have multiple mechanisms of action [4,11,26,33,34,36]. The immediate result of AChE inhibition is an increase in acetylcholine that may alter one or more steps in subsequent cholinergic pathways. For example, an increase in acetylcholine concentration may result in downregulation of, or a decrease in, cholinergic receptor number. This phenomenon is a compensatory or adaptive response by the postsynaptic cell to avoid overstimulation that could compromise an animal's ability to function normally. The number of muscarinic cholinergic receptors (MChR) is a parameter that is easily measured and can reflect changes in acetylcholine levels in tissue from an exposed animal compared to MChR in tissue from a control animal [10]. The regulation of MChRs in brain from several mammalian species following exposure to a variety of cholinesterase inhibitors has been extensively studied [3,6–10,14,17,22,23,25]. However, there are no studies reporting effects on MChR regulation in piscine species. Exposure to either carbaryl, a cholinesterase inhibitor, or permethrin, a pyrethroid which causes neurotoxicity, would be expected to increase the concentration of acetylcholine in brain synapses [4,36]. This sustained increase of acetylcholine could cause downregulation or a decrease in the number of MChR in the brain [14]. We investigated the regulation of MChR in the brain from seven species of fish, two surrogates and five listed species. Fish were classified as either cold water or warm water and were exposed to chemicals affecting cholinergic pathways (carbaryl and permethrin) and two chemicals whose effect on the cholinergic system is understood less well (4-nonylphenol and copper). The cold water species chosen were rainbow trout (*Oncorhynchus mykiss*; surrogate cold water species), apache trout (*Oncorhynchus apache*), and lahontan cutthroat trout (*Oncorhynchus clarki henshawi*). The warm water species chosen were fathead minnows (*Pimephales promelas*; surrogate warm water species), bonytail chub (*Gila elegans*), colorado squawfish (*Ptychocheilus lucius*), and razorback suckers (*Xyrauchen texanus*). We used radioligand binding to measure the number of specific MChR sites in these tissues as well as the affinity of the receptor for the specific radioligand. The object of this research was to assess if: (a) brain MChR will downregulate after 96 h of exposure to any of these compounds; and (b) effects on MChR or affinity will be similar across fish species. Our study was done in parallel with a standardized toxicity study comparing the LC_{50}'s these species [12,35].

2. Methods

2.1. Animals

Rainbow trout (RBT), apache trout (APT), lahontan cutthroat trout (LPW), bonytail chub (BTC), colorado squawfish (CSF), and razorback suckers (RBS) were obtained from various government and commercial sources as eyed eggs by the Environmental and Contaminants Research Center (ECRC). Fathead minnows (FHM) were from ECRC stocks (FHM-1 and FHM-3) or purchased commercially (FHM-2). Fish were cultured in ECRC well water until testing began. All fish were cultured at 18°C, except trout which were hatched and cultured until swim-up in 10°C well water. Fish were acclimated to ASTM reconstituted hard water for a total of 96 h prior to testing [2,37].

2.2. Toxicity testing

Static acute toxicity tests were conducted in basic accordance with EPA (660/3-75-009) and ASTM (E 729-88) procedures. Chemicals tested include carbaryl, a carbamate insecticide that inhibits cholinesterase activity, permethrin, a pyrethroid insecticide which causes neurotoxicity, 4-nonylphenol, a monoalkyl phenol which may cause narcosis and may act as an oxidative stressor and copper which alters osmoregulation. Fish were exposed, ten per jar, to 15 l of test solution in 19.6 l glass jars. Tests with cold water fish were conducted at 12°C and tests with warm water fish at 22°C. A test series consisted of five or six exposure concentrations with a 60% dilution factor. Both a solvent (acetone) control and a water control were included for each species. The maximum concentration of acetone did not exceed 0.05%. No acetone was included in the copper experiments. Each series was replicated three times within a test except when the number of fish was insufficient. Cold water exposures were: (1) LPW, and APT; (2) RBT-1; and (3) RBT-2. Warm water exposures were: (1) FHM-1, and RBS; (2) FHM-2, and BTC; and (3) FHM-3, and CSF. For a complete description of the test organisms and procedures used, see Refs. [35,12].

2.3. Tissue preparation

After 96 h exposure, surviving fish from each jar of three replicates/treatment were killed by an overdose of the anesthetic tricaine methanesulfonate (MS-222). Brains were removed from fish of a given replicate, pooled, and frozen at -80°C until the day of the assay. On the day of the assay, the tissue (≈ 100 mg) was thawed and placed in a tube with 10 ml ice-cold 50 mM Tris–HCL, pH 8.0 (Tris 8.0) buffer. The tissue was homogenized for 20 s with a tekmar tissumizer on

setting 90. Ice-cold Tris 8.0 was added to bring the final volume to 25 ml, and the samples were centrifuged at 49000 × *g* for 15 min. The supernatant was discarded, and the entire process was repeated. The resulting pellet was resuspended in 20 V (weight/volume) ice-cold ultrapure H_2O, and sample was removed for protein determination. Tris 8.0 was added to bring the final volume of the crude membrane particulate preparation to 500 V. Protein content was determined by the method of Lowry et al. [27], with bovine serum albumin as a standard.

2.4. Radioligand binding

The radioligand binding assay for MChR was performed as described by Jones and King [24]. Briefly, 970 μl of tissue homogenate were incubated in tubes with increasing concentrations of N[^{3}H-methyl]scopolamine ([^{3}H]NMS; 20 μl) for 45 min at 22°C to measure total binding. Non-specific binding was defined by [^{3}H]NMS binding in the presence of 1 μM atropine in a parallel set of tubes. Specific binding is the difference between total binding and non-specific binding. Incubations were terminated with rapid filtration through Whatman GF/B glass fiber filters. The filters were rinsed with 12 ml of ice-cold 50 mM Tris 8.0 and transferred to vials containing 10 ml scintillation cocktail (ICN Ecolume, Irvine, CA). Radioactivity was determined by scintillation spectroscopy at an efficiency of 46%.

[^{3}H]NMS was purchased from Dupont New England Nuclear (Boston, MA). Carbaryl and permethrin were donated by Rhone-Pôulenc Agricultural (Research Triangle Park, NC), and ICI Americas (Richmond, CA), respectively. 4-Nonylphenol was purchased from Fluka (New York, NY). All other drugs and buffers were purchased from Sigma Chemical (St. Louis, MO).

2.5. Data analysis

The two RBT and two of the FHM surrogate exposure values were combined whereas data from the commercially available FHM are not presented (Section 4). Binding parameters were analyzed with nonlinear regression using Graphpad InPlot® software. Statistical significance was tested with SAS one-way ANOVA using Fisher's least significant difference (LSD) and a pooled standard error. A P value of 0.05 was considered significant.

3. Results

3.1. Cold water species

Carbaryl exposure resulted in decreased numbers of MChR in RBT brain at 2.2 and 3.6 mg l^{-1} compared to control and control-acetone and 3.6 mg l^{-1} compared to all lower concentrations. This effect was not observed in brain from LPW or APT (Fig. 1), because we did not have survivors for 3.6 mg l^{-1} for either species or 2.2 mg l^{-1} for APT. There were no differences in affinity among treatments within a species except for 0.8 mg l^{-1} compared to control for LPW (Table 1).

Permethrin exposure resulted in a decreased number of brain MChR in the RBT at 3.6 μg l^{-1} compared to control and 0.8 μg l^{-1} (Fig. 1). However, the two RBT exposures were inconsistent as there was a difference between 3.6 μg l^{-1} and all lower concentrations of permethrin for RBT-1, but no significant effects of permethrin on MChR in RBT-2. LPW showed a significant decrease in brain MChR at 2.2 μg l^{-1} compared to 0.8 μg l^{-1}, but not between control and any of the other treated samples (Fig. 1). APT exposed to 1.3 μg l^{-1} permethrin had significantly fewer brain MChR than control, control-acetone or 0.8 μg l^{-1} (Fig. 1). There were no significant differences in affinity among the treatments within a species (Table 1).

Exposure to 0.22 mg l^{-1} 4-nonylphenol resulted in a decreased number of brain MChR compared to control and lower concentrations of 4-nonylphenol in RBT-2. A similar effect was observed in LPW (Fig. 2). In APT brain, exposure of 0.05 and 0.13 mg l^{-1} resulted in MChR decreases compared to control. Exposure to 0.05 mg l^{-1} copper resulted in a decreased number of brain MChR in RBT-2 compared to control, and APT at 0.05 and 0.08 mg L^{-1} compared to control, but had no significant effect in LPW brain (Fig. 2). No changes in affinity were observed in 4-nonylphenol or copper exposures (Table 1). Copper and 4-nonylphenol samples were not collected for RBT-1.

For a complete description of survival effects in these studies, see Refs. [12,35].

3.2. Warm water species

FHM exposure to 3.6 and 6.0 mg l^{-1} carbaryl resulted in a significant decrease in brain MChR compared to control and between the lower exposure concentrations and 6.0 mg l^{-1} (combined FHM-1 and FHM-3; Fig. 3). RBS exposure resulted in a significant decrease in MChR at 6.0 mg l^{-1} compared to control, control-acetone, and all lower concentrations of carbaryl. There was also a significant decrease between 3.6 and 2.2 mg l^{-1}. BTC exposure resulted in a significant decrease at 3.6 mg l^{-1} compared to all other treatments. In contrast, there was a significant increase in CSF brain MChR at 2.2 mg l^{-1} compared to control,

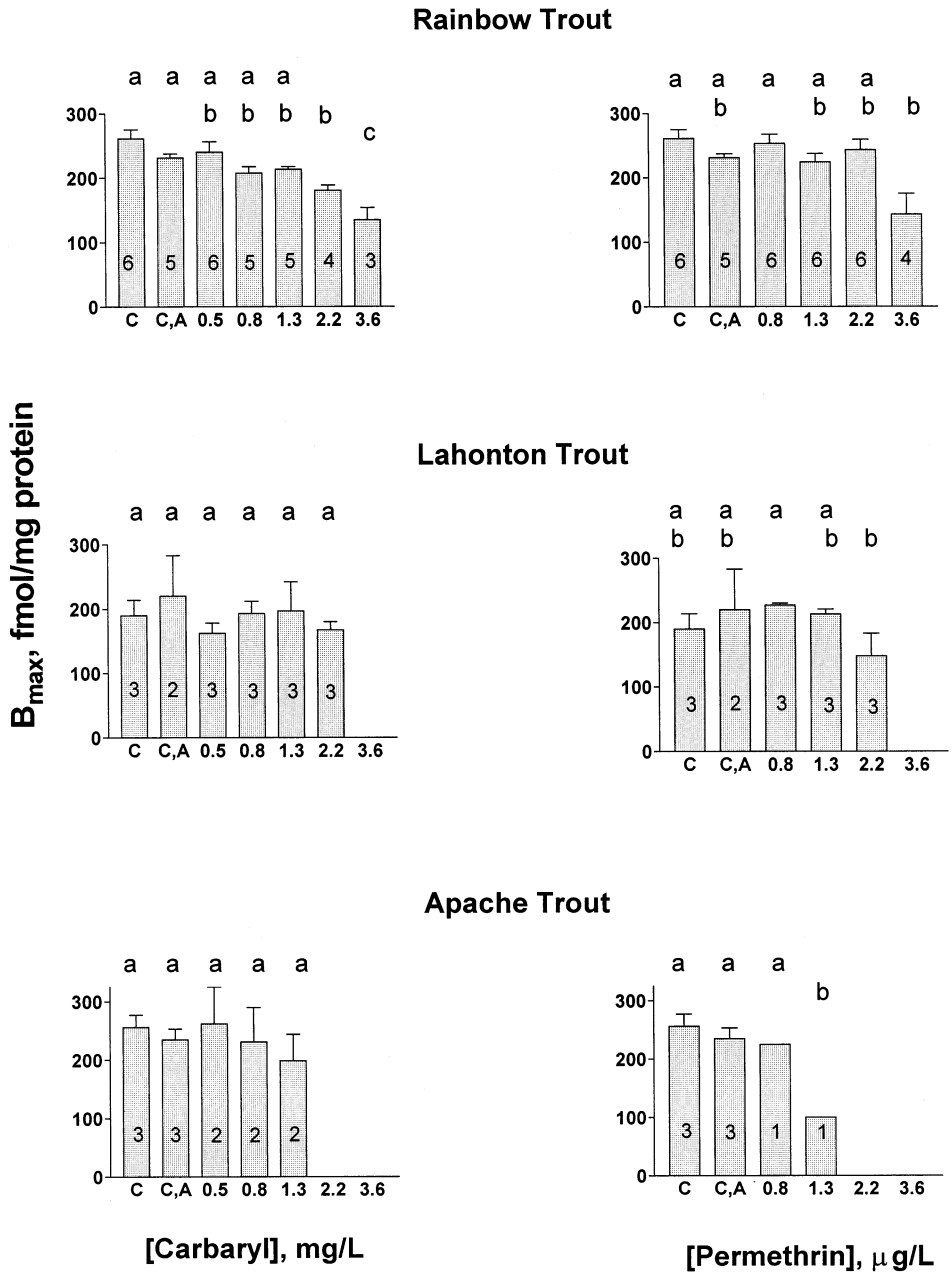

Fig. 1. The effect of carbaryl and permethrin on MChR B_{max} in cold water species. A crude membrane preparation (0.04–0.06 mg ml protein^{-1}) was incubated with a range of radioligand concentrations (10–1100 pM) with and without 1 μM atropine for 45 min at room temperature. The data are presented as fmol mg^{-1} protein and are the mean $\pm$ S.E.M. for the indicated number of pooled samples from the given exposures. Bars with different letter designations indicate significance at $P < 0.05$.

control-acetone and 1.3 mg l^{-1} (Fig. 3). There were no differences in affinity of [^{3}H]NMS for the receptor within any of the warmwater species exposed to carbaryl (Table 2).

Permethrin exposure resulted in a decreased number of brain MChR in FHM (combined FHM-1 and FHM-3) at 9 μg l^{-1} compared to control (Fig. 3). There were no survivors at higher concentrations of permethrin in FHM-1, but FHM-3 exposure also resulted in significant decreases in MChR at 15 μg l^{-1} compared to control-acetone and 25 μg l^{-1} compared to control and control-acetone, 1.9, 3.2, and 5.4 μg l^{-1} (Fig. 4). RBS exposure resulted in significant decreases in brain MChR at 9 and 15 μg l^{-1} compared to control and control-acetone and between 25 μg l^{-1} and control. There were also differences between 15, 1.9, 3.2, and 5.4 μg l^{-1}. There was a significant difference in MChR numbers between 1.9 and 3.2 μg l^{-1} permethrin in BTC, and between control and at 25 μg l^{-1} in CSF (Fig. 4). There were no differences in affinity in any of the treatments within the warm water species (Table 2).

Table 1
K_D values (nM) from [^{3}H]NMS binding in brain of the cold water species, rainbow trout, lahontan cutthroat trout, and apache trout with and without exposure to carbaryl, permethrin, 4-nonylphenol, or copper

Chemical	Rainbow trout	Lahontan cutthroat trout	Apache trout
Carbaryl (mg l^{-1})			
Control	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01
Control-A	0.09 ± 0.01	0.11 ± 0.04	0.11 ± 0.03
0.5	0.09 ± 0.01	0.11 ± 0.03	0.17 ± 0.039
0.8	0.07 ± 0.01	0.11 ± 0.01*	0.12 ± 0.02
1.3	0.08 ± 0.01	0.11 ± 0.02	0.11 ± 0.03
2.2	0.16 ± 0.09	0.11 ± 0.02	–
3.6	0.18 ± 0.08	–	–
Permethrin (μg l^{-1})			
0.8	0.09 ± 0.03	0.12 ± 0.01	–
1.3	0.07 ± 0.02	0.08 ± 0.01	–
2.2	0.08 ± 0.02	0.08 ± 0.01	–
3.6	0.08 ± 0.01	–	–
4-Nonylphenol (mg l^{-1})			
0.05	0.07 ± 0.01	0.12 ± 0.01	0.06 ± 0.01
0.08	0.06 ± 0.01	0.14 ± 0.01	0.07 ± 0.01
0.13	0.08 ± 0.02	0.14 ± 0.01	0.07 ± 0.01
0.22	–	0.12 ± 0.06	–
Copper (mg l^{-1})			
0.03	0.07 ± 0.01	0.09 ± 0.02	0.09 ± 0.01
0.05	0.08 ± 0.02	0.12 ± 0.03	0.09 ± 0.02
0.08	–	–	0.19

Composite data of mean $\pm$ S.E.M. all exposures.
* Significantly different than control.

FHM and CSF exposed to 4-nonylphenol exhibited no change in either total number of brain MChR or affinity (data not shown). Brain samples were not collected for warm water species exposed to copper.

B_{max} data for all species of warm water and cold water fish were normalized to percent of control for carbaryl and permethrin exposures to demonstrate trends among the groups (Fig. 4). Carbaryl exposure in cold and warm water fish resulted in a trend towards fewer receptors in all species except CSF for which there were no survivors at higher concentrations. A similar trend existed for all species exposed to permethrin except for BTC. However, there was inconsistency in the concentration at which downregulation occurred among species exposed to permethrin.

4. Discussion

4.1. Variability within RBT and FHM

The surrogate species chosen in these studies have been used routinely for toxicity studies [28]. In our study, we expected that the MChR binding results from each chemical exposure would be consistent for a given species. This was a correct assumption for RBT for which the results of two runs were relatively consistent. In contrast, we observed differences in the responses of FHM among the three runs. Whereas FHM-1 and FHM-3 exposures generated consistent results, FHM-2 exposure was quite different, with control values being low. One possible explanation for these results is that FHM-1 and FHM-3 were from ECRC laboratory raised stock, whereas FHM-2 was from a commercial source and may be a different strain. Due to the differences between FHM-1 and FHM-3 compared to FHM-2, data from FHM-2 were eliminated from further consideration in this study. These differences underscore the need to have a uniform source of experimental animal in multiple exposure studies if at all possible.

4.2. Overall response of all species

Not all species within a class of organisms respond similarly to a particular chemical. Some organisms may be able to overcome a chemical exposure by a compensatory response initiated by the exposure. We used downregulation of MChR to assess compensatory ability among several species exposed to a series of chemicals. The overall response among species was relatively consistent in these studies, but there were exceptions. For example, carbaryl or permethrin exposure resulted in decreases in receptor number at higher concentra-

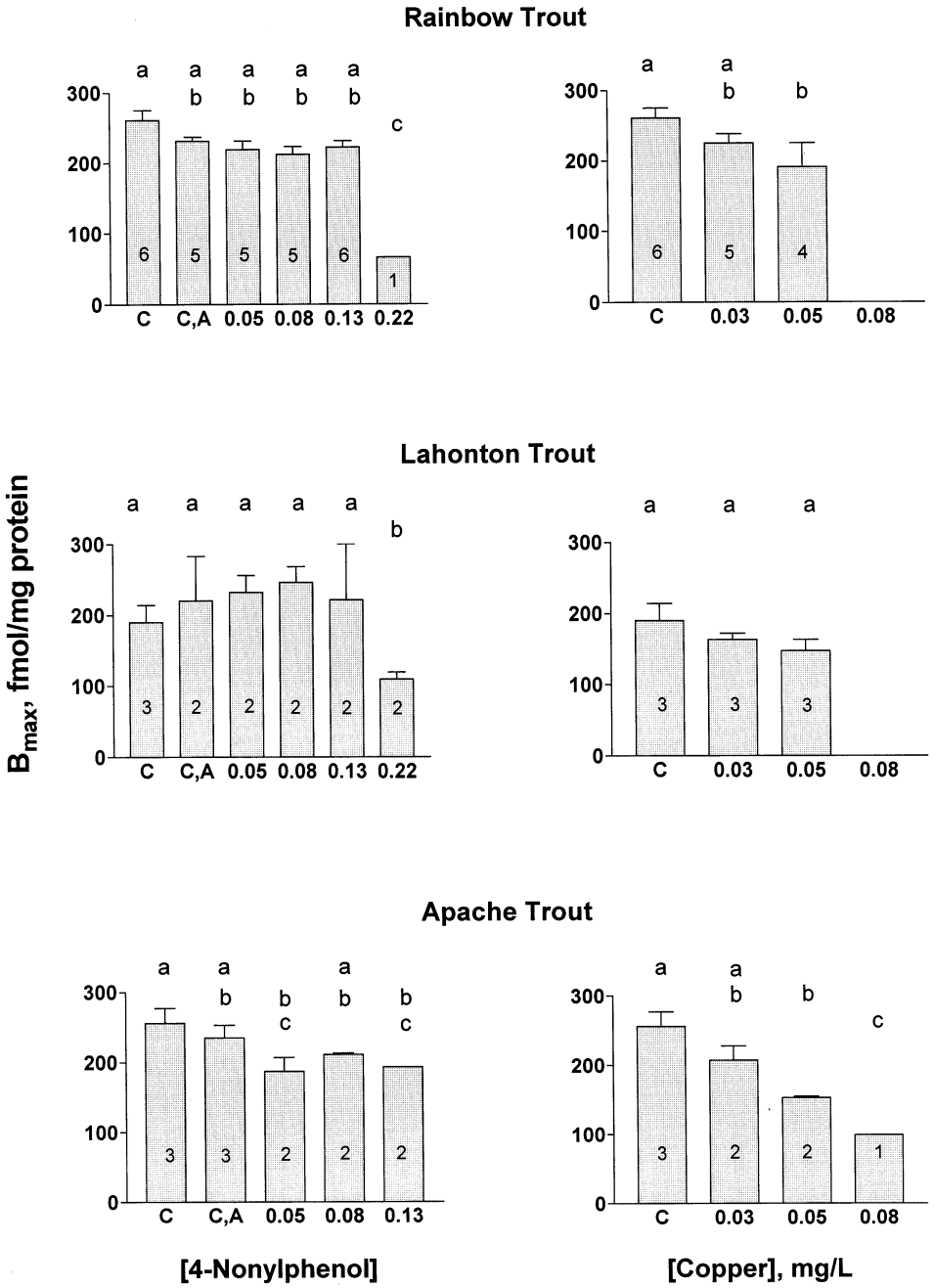

Fig. 2. The effect of copper and 4-nonylphenol on MChR B_{max} in cold water species. Conditions were similar to that described in Fig. 1. The data are presented as fmol mg protein^{-1} and are the mean $\pm$ S.E.M. for the indicated number of pooled samples from the given exposures. Bars with different letter designations indicate significance at $P < 0.05$.

tions in the majority of species, but not all. In addition, in some species receptor numbers in the control-acetone treatment were consistently lower, though not statistically significant, than control values. Presumably, acetone could alter membrane fluidity and thus alter binding parameters. However, there were no differences in MChR affinity between control and control-acetone in any species in this study which we might expect if membrane fluidity was causative. Huang et al. [21] demonstrated this effect of monoketones on DHA binding (β-adrenoceptors) in mouse synaptosomes and found where there were no effects on receptor number, but did observe decreased affinity. If our results simply were due to an acetone effect on receptor number, however, similar results would be expected across species and across concentrations of chemicals. Our observations do not support this statement as we did not observe this phenomenon in all species.

Table 2
K_D values (nM) from [^{3}H]NMS binding in brain from the warm water species, fathead minnows, razorback suckers, bonytail chub, and colorado squawfish with and without exposure to carbaryl, permethrin, or 4-nonylphenol

Chemical	Fathead minnows	Razor back suckers	Bonytail chub	Colorado squawfish
Carbaryl (mg l^{-1})				
Control	0.17 ± 0.04	0.17 ± 0.05	0.10 ± 0.03	0.10 ± 0.01
Control-A	0.12 ± 0.04	0.16 ± 0.04	0.08 ± 0.01	0.10 ± 0.01
0.8	0.19 ± 0.08	0.14 ± 0.03	0.08 ± 0.01	0.09 ± 0.03
1.3	0.21 ± 0.08	0.13 ± 0.01	0.07 ± 0.01	0.09 ± 0.01
2.2	0.10 ± 0.02	0.16 ± 0.02	0.07 ± 0.01	0.07 ± 0.01
3.6	0.10 ± 0.02	0.17 ± 0.04	0.07	0.06 ± 0.01
6.0	0.15 ± 0.05	–	–	–
Permethrin (μg l^{-1})				
1.9	0.14 ± 0.06	0.14 ± 0.06	0.10 ± 0.03	0.09 ± 0.03
3.2	0.09 ± 0.01	0.15 ± 0.04	0.09 ± 0.02	0.08 ± 0.01
5.4	0.12 ± 0.02	0.14 ± 0.04	0.08 ± 0.04	0.10 ± 0.01
9.0	0.10 ± 0.01	0.20 ± 0.05	0.06 ± 0.01	0.12 ± 0.02
15.0	0.11 ± 0.02	0.31	–	0.18 ± 0.08
25.0	0.11 ± 0.01	0.18	–	0.18 ± 0.08
4-Nonylphenol (mg l^{-1})				
0.08	0.10 ± 0.01	–	–	0.09 ± 0.01
0.13	0.08 ± 0.01	–	–	0.10 ± 0.02
0.22	0.10 ± 0.01	–	–	0.16 ± 0.05

Composite data of mean $\pm$ S.E.M. for all exposures.

APT survivors at higher concentrations of carbaryl making comparisons with other species difficult in these studies. A possible explanation is that the two listed species are more sensitive to carbaryl and were unable to adapt to the carbaryl-induced increase in brain acetylcholine concentration. The carbaryl LC_{50}'s at 96 h for these three species (RBT, LPW, and APT) were not statistically different, arguing against differences in lethal sensitivity, at least at 50% [35]. However, the fact that there were no survivors at higher concentrations suggests that adaptation to carbaryl exposure may occur less readily in LPW and APT, potentially compromising these species.

Permethrin exposure resulted in MChR downregulation in FHM and RBS brain, but the concentrations required for observation of this phenomenon were much greater than that observed in cold water species. In addition, there were differences within FHM between exposures for each of these chemicals. Each of the cold water species demonstrated a decrease in MChR number following permethrin exposure, but at different concentrations. For example, RBT-1 showed no effect on receptor number except at 3.6 μg l^{-1} whereas, APT receptor number decreased at 1.3 μg l^{-1}. These differences in relative sensitivity to permethrin between APT and RBT are consistent with the toxicity data in which the 96 h LC_{50} for APT is lower than RBT (1.71 versus 3.31 μg l^{-1}, $P = 0.08$; 12). The inconsistency in response in these species may occur because permethrin may cause different levels of acetylcholinesterase inhibition among species that would result in inconsistent levels of acetylcholine in brain synapses [33,34]. Such a difference could affect not only the exposure concentration at which downregulation occurs, but whether or not downregulation would occur at all. In addition, the multiple mechanisms of action of permethrin could also affect cholinesterase activity in a less direct manner. For example, pyrethroid insecticides have been shown to indirectly inhibit GABA-dependent chloride channels in trout brain [15]. An alteration in the major inhibitory neurotransmitter in the brain, could have dramatic effects on excitatory cholinergic pathways. Another possibility is that permethrin's lipophilicity causes a generalized perturbation of the lipid membrane reflected by allosteric changes in membrane proteins such as receptor molecules. Moya-Quiles et al. [29] have shown such an effect of permethrin on membrane fluidity in multilamellar liposomes. However, if membrane perturbation resulted in different sensitivities, we would have expected to see changes in affinity, and we did not.

Differences in brain MChR number between control and copper-exposed animals were somewhat surprising. At the concentrations of copper used in these experiments, we did not expect to see a decrease in receptor number. However, Hogan and Knowles [20] have reported an acetylcholinesterase inhibition of greater than 90% with in vitro studies of blue gill and channel catfish brain homogenates. Similar effects were observed in cutthroat trout [19]. In each of these studies, 1 mM copper was used which is 100 times as much as was used in our studies. Whereas, a similar decrease in

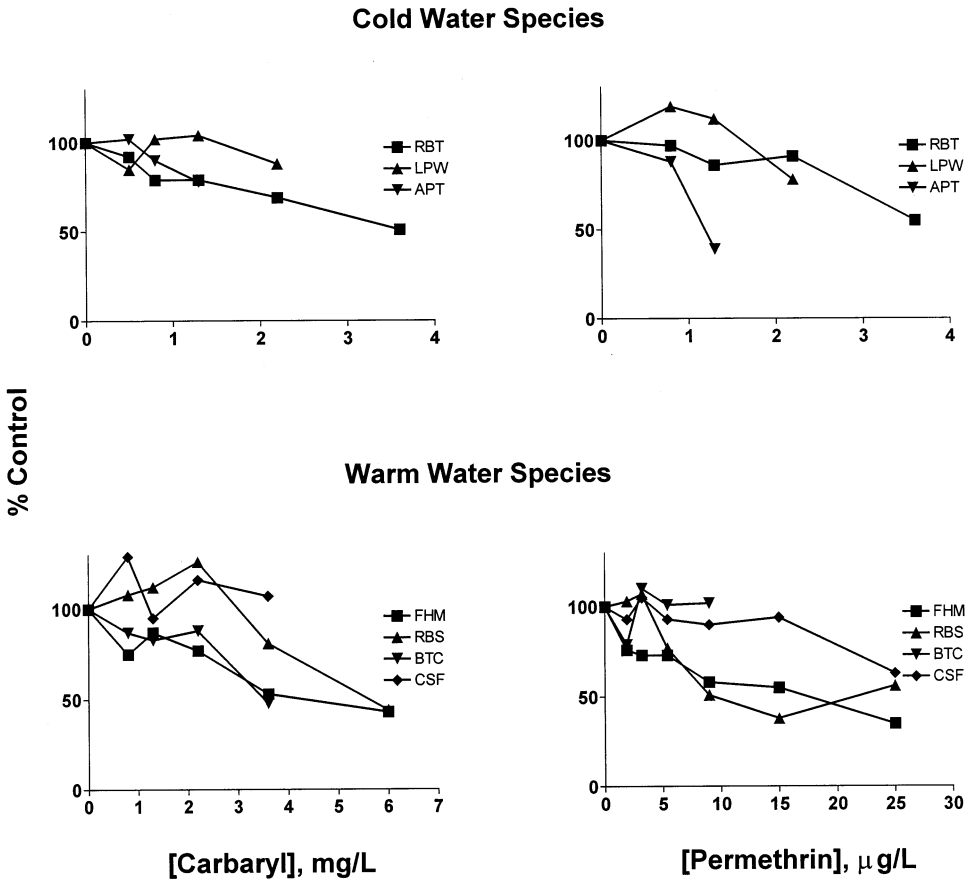

Fig. 4. Composite of data from cold water and warm water species exposed to carbaryl and permethrin. The data are presented as percentage of control B_{max} for each species. Error bars are not included for clarity of figure. The data from which this figure was generated are shown in Figs. 1 and 3 as well as the text.

MChR number as our results has been demonstrated in various rat brain regions in in vitro binding studies [16], it would appear that low level copper has dramatic effects on the cholinergic system, probably through inhibition of acetylcholinesterase. The copper concentration we used in our exposure studies was 0.5–1.25 μM which is similar to 3μM used in the rat study.

We have found no published information regarding the possible effects of 4-nonylphenol on neurotransmitter receptors of any kind. One interpretation of our data is that 4-nonylphenol caused a generalized toxicity which was reflected by the decrease in receptor number. This generalized toxicity could involve membrane perturbation such that binding would be altered. However, we would have expected a change in affinity if membrane perturbation was the causal factor which we did not observe.

4.4. Summary

The results of this study demonstrate a trend in downregulation of brain MChR in most species exposed for 96 h to a cholinesterase inhibitor, carbaryl, as well as a pyrethoid with multiple mechanisms of action, permethrin. There was some variability in the concentration at which downregulation was apparent across species. With the exception of permethrin exposure in APT, however, our results indicate that surrogates are useful in assessing sublethal physiological responses to chemicals with a known mechanism of action and support their use for assessing physiological responses of chemicals with diverse, less clear mechanisms of action such as permethrin and copper.

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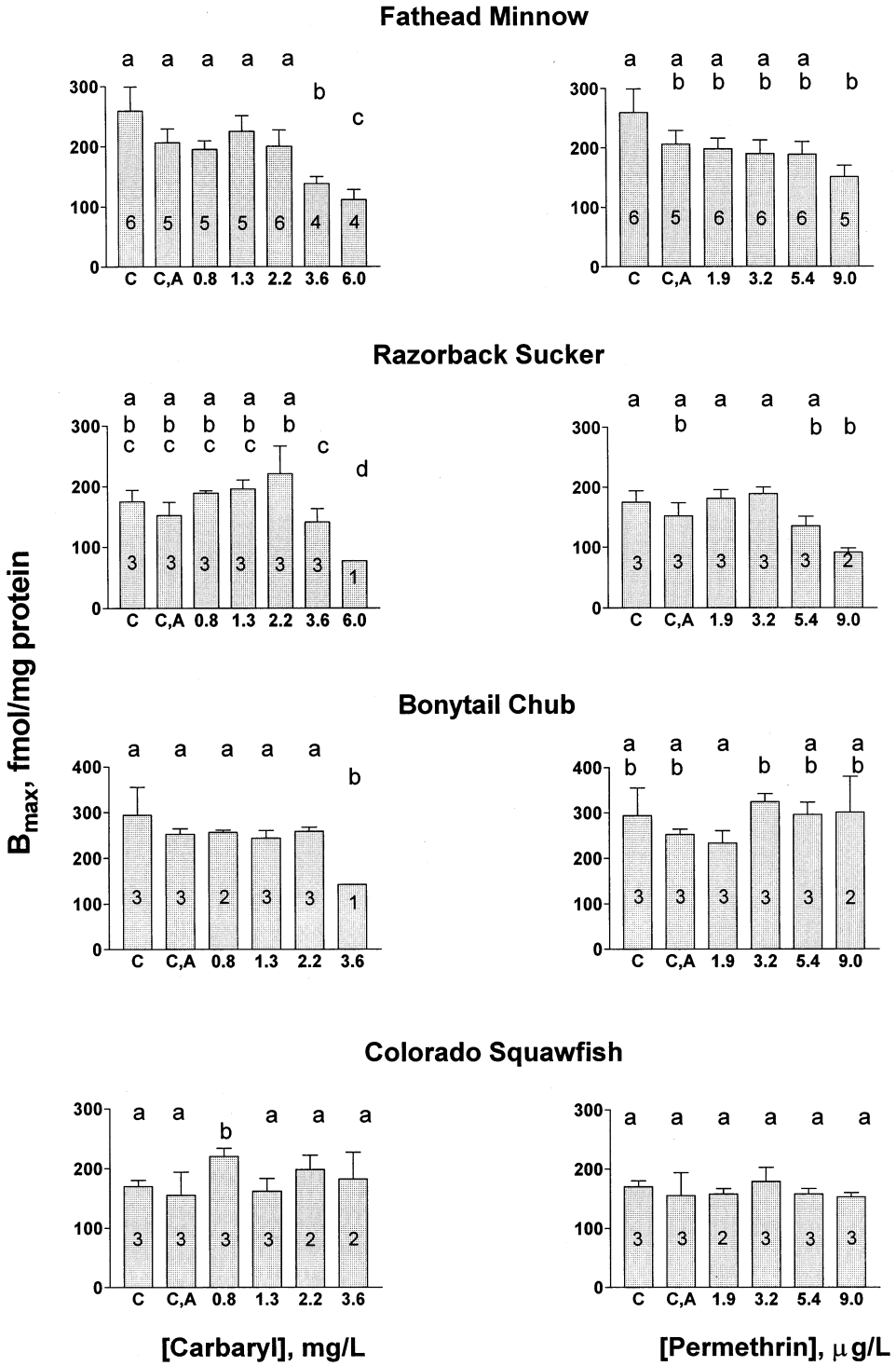

Fig. 3. The effect of carbaryl and permethrin on MChR B_{max} in warm water species. Conditions were similar to that described in Fig. 1. The data are presented as fmol mg protein^{-1} and are the mean ± S.E.M. for the indicated number of pooled samples from the given exposures. Bars with different letter designations indicate significance at $P < 0.05$.

4.3. Cold versus warm water responses

Generally, the warm water species have been less sensitive to all chemicals than the cold water fish in toxicity studies based on overt lethality [35]. However, downregulation of MChR in carbaryl exposed animals occurred in all warm water species except CSF at concentrations similar to that observed in RBT. In RBT, higher concentrations of carbaryl resulted in downregulation of the MChR. There were no LPW or