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Changes in Neurotransmitter Levels in Channel Catfish After Exposure to Benzo(a)pyrene, Naphthalene, and Aroclor 1254

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Several pollutants have been found to alter the levels of brain neurotransmitters. In some investigations all the neurotransmitters studied were affected (TAYLOR and DISTEFANO 1976, MCDONALD 1979, SHARMA 1973, HEINZ 1980), while in other studies only some of the neurotransmitters were affected (HRDINA 1973, SHARMA 1976, FINGERMAN and RUSSELL 1980). In general, when norepinephrine (NE) and dopamine (DA) were affected, their levels decreased while in studies where 5-hydroxytryptamine (5-HT) was affected there was no consistency in the direction of change. In some species 5-HT was elevated and in others it was depressed. The effects of chemicals on neurotransmitters are probably species and dose-related, making correlations difficult among studies using different species of animals and different dose rates. In addition, the effects on animal physiology by chemical pollutants in the environment are further complicated by the fact that in nature animals are rarely exposed to a single pollutant. Consequently, experiments were conducted to determine the effects of a single exposure to the common environmental contaminants benzo(a)pyrene (BP), naphthalene (N), and the PCB Aroclor 1254, not only alone but also in combination, on the neurotransmitters 5-HT, NE and DA in the brain of the channel catfish (Ictalurus punctatus), a species that has not been studied previously in this regard.

MATERIALS AND METHODS

Specimens of young channel catfish, Ictalurus punctatus, weighing 30 to 50 g, were purchased from a commercial fish farm in Oklahoma, for use in these experiments. The stock supply of fish was maintained caged in a pond and fed a commercial catfish food daily. Fish were transported to the laboratory and placed in aerated glass aquaria under static conditions the day before each experiment was to begin. Water temperature in the aquaria was 19 °C. The fish were not fed from the time they were first selected from the pond for use in the laboratory.

Practical grade 3,4-benzo(a)pyrene (Lot Number 79C-05252) (Sigma) and Aroclor 1254 (Lot Number H116A) (Analabs) were used. One hundred mg of benzopyrene (BP), Aroclor 1254 (PCB), or naphthalene (N) were dissolved in one ml corn oil for injection into the fish. Fish were injected intraperitoneally with 100 µg BP, PCB or N, singly and in combinations, per gram body weight. Control fish received only corn oil (1 µl/g). The fish were sacrificed at 9 a.m. by

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cutting the spinal cord at the base of the skull and then removing the brain. Assays for brain 5-HT, NE and DA were conducted following the methods of MAICKEL et al.(1968) and ANSELL and BEESON (1968). Statistical analyses involved calculations of standard error of the mean and Student's *t*. Significance was set at the 95% confidence interval.

EXPERIMENTS AND RESULTS

Experiments were designed to determine the effects of one i.p. injection of PCB, BP and N, singly and in combinations, on the brain neurotransmitters of *Ictalurus punctatus*. The catfish were injected with BP, PCB, N, PCB + BP, PCB + N or N + BP at 9 a.m. and brains were removed 24 and 48 h later. The experiments were repeated twice.

After 24 h, the 5-HT levels in the groups injected with BP and PCB were not significantly different from the controls (TABLE I). However, the 5-HT levels were significantly greater than the controls in the groups given N ($p<0.05$), PCB + N ($p<0.01$), N + BP ($p<0.02$), and PCB + BP ($p<0.001$). Norepinephrine levels were significantly different from the controls in the brains of fish injected with N + BP ($p<0.05$) and PCB + BP ($p<0.02$), with the N + BP NE level being greater than in the controls, the group receiving PCB + BP being less. Norepinephrine levels were not significantly different from the control level in the other groups, BP, PCB, N, PCB + N. Dopamine levels were not significantly different from the controls in the groups given BP and PCB. Dopamine levels were significantly higher than the controls in the other chemical-injected groups, N ($p<0.001$), PCB + N ($p<0.001$), N + BP ($p<0.001$), PCB + BP ($p<0.001$).

TABLE I

Averages (mean ± standard error) of brain neurotransmitters in *Ictalurus punctatus* 24 h after exposure to PCB, BP and N. Number shown in parentheses. * Statistically significant ($p \leq 0.05$).

	5-HT	NE	DA
Controls	0.47±0.05 (10)	0.47±0.09 (10)	0.16±0.04 (9)
BP	0.54±0.07 (10)	0.61±0.14 (10)	0.18±0.06 (10)
PCB	0.32±0.07 (8)	0.55±0.14 (10)	0.08±0.01 (9)
N	0.60±0.08* (9)	0.47±0.04 (9)	0.58±0.06* (9)
PCB + N	0.79±0.09* (11)	0.51±0.07 (11)	0.71±0.08* (10)
N + BP	0.68±0.06* (10)	0.61±0.03* (8)	0.42±0.05* (10)
PCB + BP	0.85±0.06* (10)	0.32±0.06* (10)	0.68±0.05* (10)

After 48 h, 5-HT levels were significantly lower in the BP-injected group ($p<0.02$) and significantly higher in groups given N ($p<0.05$), N + BP ($p<0.01$), and PCB + BP ($p<0.01$) than in the controls (TABLE II). Levels of 5-HT in groups injected with PCB and PCB + N were not significantly different from controls. Norepinephrine levels in the groups injected with N, PCB + N, and N + BP were all significantly greater than in the controls (N, $p<0.001$; PCB + N, $p<0.01$; N + BP, $p<0.001$), while the NE level was significantly lower in the PCB + BP group ($p<0.05$). Norepinephrine levels of groups given BP and PCB were not significantly different from the NE level of the controls. After 48 h, DA levels were significantly different in only two of the chemical-injected groups. The group given N had DA levels significantly lower than in the control ($p<0.05$), while the group given PCB + BP was significantly higher ($p<0.001$). The groups receiving BP, PCB, PCB + N, and N + BP had DA levels that were not significantly different from controls.

TABLE II

Averages (mean ± standard error) of brain neurotransmitters in *Ictalurus punctatus* 48 h after exposure to PCB, BP and N. Number shown in parentheses. *Statistically significant ($p \leq 0.05$).

	5-HT	NE	DA
Controls	0.48±0.05 (17)	0.51±0.07 (17)	0.19±0.06 (17)
BP	0.25±0.05* (9)	0.39±0.04 (9)	0.32±0.15 (9)
PCB	0.35±0.05 (11)	0.53±0.10 (11)	0.14±0.07 (11)
N	0.70±0.11* (10)	1.16±0.15* (10)	0.06±0.01* (10)
PCB + N	0.54±0.07 (10)	0.92±0.12* (10)	0.10±0.01 (10)
N + BP	0.68±0.07* (10)	1.29±0.24* (10)	0.19±0.04 (10)
PCB + BP	0.79±0.10* (11)	0.32±0.05* (10)	0.49±0.05* (10)

DISCUSSION

The chemicals tested affected levels of brain neurotransmitters, but the effect showed no discernible pattern. The neurotransmitters affected by a given chemical may vary from species to species and in some cases the direction of the effect on a single neurotransmitter may be reversed with longer exposure. In the present study, N, singly and in combination with BP and PCB, affected the neurotransmitters significantly, causing changes in 5-HT and NE levels after 24 or 48 h. N, administered singly, caused an increase

in DA at 24 h but a decrease at 48 h. Reversal of the effect of a chemical on brain levels of a neurotransmitter over time has been previously reported. TAYLOR and DISTEFANO (1976) found that sublethal doses of methylmercury produced an initial decrease in brain neurotransmitters in rats, but increased levels by the 15th day of exposure. When Fundulus grandis was exposed to N the DA level was decreased after 24 h (RUSSELL 1980). Thus it appears that responses of fish to N varies from species to species and over time.

The time course of the effect of chemicals on brain neurotransmitters is probably due to the rate of uptake of the pollutant and the effect the pollutant is exerting on the synthesis, storage and/or release of individual neurotransmitters. In addition, several investigators have suggested that the capacity of the brain to take up pollutants, metabolize them, and flush them out, is very different from other tissues such as the liver (COLLIER et al. 1980, DIXIT and ANDERSON 1977, PETERSON and GUINEY 1979).

The effects of combinations of chemicals does not appear to be predictable from the effects of the individual pollutants. In the present study, though BP and PCB administered singly had little or no effect (except BP on 5-HT after 48 h) on the brain neurotransmitters, BP + PCB affected them significantly at both time periods by increasing the levels of 5-HT and DA and decreasing NE. In several instances in TABLES I and II, it can be seen, as with BP and PCB on 5-HT after 24 h, that the change in the level of neurotransmitter in a group receiving a combination of chemicals was greater than in groups receiving either chemical alone. Some chemicals in combination perhaps interact to affect the uptake of one or both chemicals by the brain, the length of time the chemicals would ordinarily remain in the brain, or the influence of one or both chemicals on synthesis, storage or release of neurotransmitters. Study of the mechanisms by which combinations of two pollutants show effects on neurotransmitters that differ from those produced by either alone are beyond the scope of this paper. However, it is clear that in order to gain a more accurate understanding of what is happening in the environment where several pollutants may exist in biologically significant concentrations, physiological responses of subject species must be studied after exposure to combinations of chemicals as well as individual chemicals.

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