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Neurochemical Effects of Cyclopiazonic Acid in Chickens (42673)

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Abstract. Chickens dosed (per os) with cyclopiazonic acid (CPA) at 0.5, 5.0, and 10 mg/kg body weight showed significant ($P \leq 0.05$) increases in brain dopamine and serotonin concentrations 96 hr after dosing. The increases coincide with significant decreases in homovanillic acid and subtle increases of dihydroxyphenylacetic acid and 5-hydroxyindoleacetic acid concentrations. The elevated dihydroxyphenylacetic acid and 5-hydroxyindoleacetic acid concentrations may be related to the elevated concentrations of dopamine and serotonin, respectively. The observed changes in neurotransmitter/metabolite concentrations 96 hr after dosing parallel elimination of CPA from the birds skeletal muscles; however, they do not correlate with the significant weight losses in these birds at 48 and 96 hr after dosing. The brain weights of the treated birds were statistically insignificant from their respective controls, although increases in brain weight–body weight ratio within treatments and with time correlated with CPA toxicity. No significant changes were observed in dopamine, dihydroxyphenylacetic acid, homovanillic acid, serotonin, and 5-hydroxyindoleacetic acid concentrations among the treatments at 3, 24, and/or 48 hr after dosing.

Cyclopiazonic acid (CPA) (1), a mycotoxin found in agricultural commodities, i.e., cheese (2), corn (3), peanuts (4), stored grains (5), cereal products (6), and fermented sausages (7), has been shown to accumulate in skeletal muscles of rats (8) and chickens (9). In addition, CPA in mice produced hypokinesia, catalepsy, hypothermia, opisthotonus, and atypical convulsions (10). Subsequently, these behavioral observations have been associated with changes in the neurotransmitter and metabolite concentrations of dopamine, homovanillic acid, dihydroxyphenylacetic acid, and 5-hydroxyindoleacetic acid (11). Behavioral changes and the neurological signs of opisthotonus have led some researchers to suggest (12) CPA may have been a major contributor (along with aflatoxins) to the Turkey X disease which killed 100,000 turkeys in England in 1961 (13).

Since CPA's toxicity varies with the species (12, 14) and has been shown to accumulate in the edible meat of poultry (9), the objective of this study was to investigate CPA's induced behavioral changes in poultry, and correlate these changes with neurochemical concentrations previously observed in mice (11).

Materials and Methods. Cyclopiazonic acid was obtained as previously described (15). Four-week-old (unsexed) chickens (*Gallus gallus,* Peterson:Arbor Acre Cross) were dosed (per os) with 0.5, 5.0, and 10.0 mg/kg body weight or alone with solvent vehicle (1.0 *N* $NaHCO_3$, 2 ml/kg) and decapitated 3, 24, 48, or 96 hr after dosing (9). The brains were immediately removed over ice, weighed, and stored in 0.5 *N* $HClO_4$–1% cysteine solution at −80°C until analyzed for dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5HT), and 5-hydroxyindoleacetic acid (5HIAA) by high-performance liquid chromatographic–electrochemical analysis. Standards, instrumentation, mobile phase, procedures, and statistical analyses were as previously described (11, 16). Because of unidentified interferences from chicken whole brain homogenates, norepinephrine (NE), epinephrine (E), and dihydroxyphenylalanine (DOPA) could not be resolved or quantitated.

Results. Except for the increases in HVA concentrations in the 10 mg/kg CPA-treated birds (Table I), no other significant differences were observed (as compared to their controls) in the neurochemicals, the brain

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TABLE I. BRAIN NEUROTRANSMITTER-METABOLITE CONCENTRATIONS (ng/g BRAIN TISSUE) IN CHICKENS DOSED (po) WITH CPA[1]

Time	n[2]	ng/g brain tissue (±SD)[3]				
		DA	DOPAC	HVA	5HT	5HIAA
3 hr						
0	5	95 (5)^{a,b}	69 (12)	107 (14)^{c}	592 (18)	229 (30)
0.5	10	105 (17)^{b}	59 (17)	114 (17)^{c,d}	575 (38)	240 (41)
5.0	10	102 (15)^{a,b}	65 (10)	113 (19)^{c,d}	579 (48)	250 (48)
10.0	10	87 (13)^{a}	74 (17)	129 (23)^{d}	585 (40)	251 (45)
24 hr						
0	5	110 (25)	64 (9)	108 (14)	596 (59)	224 (31)
0.5	10	107 (17)	55 (11)	114 (19)	577 (72)	260 (23)
5.0	10	105 (27)	59 (13)	123 (44)	522 (127)	258 (74)
10.0	10	117 (32)	59 (15)	127 (19)	578 (63)	232 (40)
48 hr						
0	5	116 (17)	63 (13)	125 (32)	621 (71)	267 (50)
0.5	10	111 (30)	69 (15)	119 (17)	605 (93)	254 (66)
5.0	10	107 (40)	62 (15)	129 (46)	647 (114)	245 (62)
10.0	10	113 (34)	64 (15)	118 (33)	575 (83)	206 (69)
96 hr						
0	5	128 (12)^{e}	72 (12)^{g}	117 (24)^{i}	515 (31)^{k}	238 (38)^{m}
0.5	10	163 (22)^{f}	80 (9)^{g,h}	92 (15)^{j}	569 (36)^{l}	263 (23)^{m,n}
5.0	10	163 (25)^{f}	83 (6)^{h}	96 (23)^{j}	595 (35)^{l}	298 (44)^{n}
10.0	10	173 (32)^{f}	79 (11)^{g,h}	98 (11)^{j}	555 (32)^{l}	249 (18)^{m,n}

[1] DA, dopamine; DOPAC, dihydroxyphenylacetic acid; HVA, homovanillic acid; 5HT, serotonin; 5HIAA, 5-hydroxyindoleacetic acid.
[2] n = number of chickens/dose.
[3] Values with different superscripts vary significantly at $P \leq 0.05$.

weights (Table II), and/or the brain weight–body weight ratios 3 hr after drug administration. The DA concentrations in the 10 mg/kg-treated birds (3 hr, Table I) were no different from those of the controls or the birds given 5.0 mg/kg treatments, but these concentrations were significantly different ($P \leq 0.05$) from those of the 0.5 mg/kg treatments. In this same time element, DA in the controls and the 0.5 and 5.0 mg/kg doses were statistically insignificant ($P \leq 0.05$).

Twenty-four hours after CPA administration, the only observed differences ($P \leq 0.05$) in the treated birds when measured against their controls were reduced brain weights in those birds administered 0.5 mg/kg CPA (Table II), which coincided with the reduced brain weight–body weight ratio (Table III). However, there were no significant differences in the brain weights among the 0.5, 5.0, and 10 mg/kg-dosed birds, as there were no differences among the controls and those given 5.0 and 10.0 mg/kg treatments. The brain weight–body weight ratio (Table III)

TABLE II. BRAIN WEIGHTS OF CHICKENS DOSED (po) WITH CYCLOPIAZONIC ACID

Dose of CPA (mg/kg)	n[1]	Brain weights, mg (±SD)			
		3 hr	24 hr	48 hr	96 hr
0	5	1938 (103)	2022 (98)^{a}	2065 (48)^{c}	2100 (129)
0.5	10	2019 (71)	1898 (138)^{b}	2023 (143)^{c,d}	2182 (137)
5.0	10	1928 (127)	1966 (87)^{a,b}	2067 (107)^{c}	2179 (114)
10.0	10	1953 (84)	1982 (71)^{a,b}	1922 (131)^{d}	2128 (96)

[1] n = number of chickens/dose/treatment.
[a–d] Different superscripts differ significantly at $P \leq 0.05$.

TABLE III. RATIO BRAIN WEIGHT–BODY WEIGHT

Dose of CPA (mg/kg)	n[1]	(mg/g ± SD) 3 hr	24 hr	48 hr	96 hr
0	5	3.59 (.39)	3.64 (.15)[a]	3.21 (.21)[d]	3.05 (.39)[g]
0.5	10	3.75 (.36)	3.27 (.37)[b]	3.13 (.31)[d]	3.16 (.43)[g]
5.0	10	3.89 (.36)	3.63 (.29)[a]	3.60 (.43)[e]	3.28 (.33)[g,h]
10.0	10	3.56 (.27)	4.05 (.39)[c]	4.12 (.37)[f]	3.60 (.44)[h]

[1] n = number of chickens/dose/treatment.
[a–h] Different superscripts differ significantly ([a–f], $P \leq 0.01$; [g–h], $P \leq 0.05$).

was elevated in the 10 mg/kg treatments 24 hr after CPA and this elevation was significantly different ($P \leq 0.01$) from the controls and the 0.5- and 5.0 mg/kg-dosed birds.

Forty-eight hours after CPA administration, there were no differences in the neurotransmitter–metabolite concentrations (Table I) and brain weights (Table II) of the treated birds. The brain weight–body weight ratios in those given 5.0 and 10.0 mg/kg treatments were significantly elevated ($P \leq 0.01$) above those of the controls and the 0.5 mg/kg dosed birds. In addition, this ratio is significantly different between the 5.0 and the 10.0 mg/kg treatments.

Ninety-six hours after CPA administration, the DA and 5HT concentrations in the 0.5, 5.0, and 10.0 mg/kg CPA-treated birds were significantly elevated above those of their controls (Table I). HVA levels were depressed in the same treatments. Those birds dosed with 5.0 mg/kg CPA had elevated DOPAC concentrations (compared to those of their controls); however, these differences were not significant for the 0.5 and/or the 10.0 mg/kg dosed birds. Also the DOPAC concentrations in the controls were not different ($P \leq 0.05$) from those in the groups given 0.5 and 10 mg/kg doses. The concentration of 5HIAA in the 5.0 mg/kg group was elevated as compared to that of the controls, but there were no differences among the 0.5, 5.0, and 10.0 mg/kg dosed birds. In addition, there were no differences among the controls and 0.5 and 10.0 mg/kg dosed birds. The brain weights did not differ significantly 96 hr after CPA; however, the brain weight-body weight ratio in the 10.0 mg/kg treated birds was elevated ($P \leq 0.05$) above those of the controls and the 0.5 mg/kg treated birds. There were no differences ($P \leq 0.05$) between treatments at 5.0 and 10.0 mg/kg dosages nor were there any differences among the controls and the 0.5 and 5.0 mg/kg dosed birds.

Discussion. Thirty minutes after CPA injection (ip), Nishie *et al.* (11) observed that the DA increases in mouse forebrain and hindbrain coincide with increases in both DOPAC and HVA concentrations (hindbrain) and decreases in 5HIAA concentrations (forebrain and hindbrain). These changes in neurotransmitter–metabolite(s) levels correlated with behavioral changes in the animals tested (i.e., catelepsy, reduced body temperature, and spontaneous motor activities). No changes were observed 24 hr after dosage except for increases in NE and HVA concentrations in the hindbrain.

In the current investigations on chickens, except for the birds exhibiting a somewhat subdued behavior and in the high-dosed birds an unthrifty condition 96 hr after dosing (9), none of the birds had behavioral signs indicative of CPA toxicity at any of the levels tested. This would suggest either that 4-week-old chickens have a greater tolerance for the neurotoxic effects of CPA than mice or rats or that there are differences in metabolism of CPA among species. The elevated concentrations of DA in the 0.5, 5.0, and 10.0 mg treatments at 96 hr after dosing (Table I) coincide with decreased concentrations of HVA and subtle (insignificant) increases in DOPAC levels. Elevated 5HT levels in the 0.5, 5.0, and 10.0 mg CPA-treated birds at 96 hr (Table I) coincide with a subtle increase in 5HIAA concentrations.

The differences observed in HVA concentrations for the 10.0 mg treatments 3 hr after

CPA administration (Table I) and the brain weights in the 0.5 mg treatments 24 hr after CPA administration may be attributed to standard biological variations and/or differences expected in a random population of unsexed birds. It is unknown at present if the observed changes in DA, HVA, and 5HT concentrations at 96 hr for 0.5, 5.0, and 10.0 mg treatments (Table I) are due to neurotoxicity of CPA (or its metabolites). Predicated on these investigations and those of Norred *et al.* (8, 9), Nishie *et al.* (10, 11, 17), and Morrissey *et al.* (14), the neurochemical changes observed may result from functional changes involving the muscles (smooth, striated, and cardiac) (17). However, the differences observed in the concentrations of DA and 5HT do coincide with the subdued behavior in those birds 96 hr after CPA administration. Spooner and Winters (18) have shown in chicks that 5HT (sc) produced EEG patterns indicative of sleep with behavior described as sleep-like. In addition, DA yielded alert (arousal) EEG patterns followed by sleep-like behavior. The same sleep-like behavior was rapidly produced ($\leq$2 min) by administering NE. It was thus concluded that the turnover of excess DA to NE is what induced the onset of alertness followed by sleep-like behavior. Nishie *et al.* (10) described similar EEG patterns in rabbits dosed with CPA (i.e., sedation with activated EEG).

Even though CPA elimination patterns analogous to those for chickens (9), have been reported for rats (8), the absorption, metabolism, and subsequent behavioral signs of toxicity in the rat may be a more pronounced syndrome. Nevertheless, the subdued behavior observed in these birds coincides with elevated brain DA and 5HT concentrations 96 hr after CPA administration. Unfortunately, due to extraneous materials in chicken whole brain homogenates, NE could not be quantitated (in this mobile phase or column) and subsequently compared with the elevated DA concentrations. Four-week-old chickens have an immature, rapidly developing blood brain barrier (18–20). Therefore, low-molecular-weight electroactive compounds produced during development and final maturation of the functional blood brain barrier could account for these interferences. These problems were not encountered with previous studies (11, 16) using mature mice or rats, and are the subject of future investigations.

Over the study period (i.e., 96 hr) brain weight increased ($\geq$8%, Table II), with the brain weight–body weight ratio reaching a peak within 24 hr (Table III). This observation is in agreement with previous investigations (21) that the 4- to 7-week-old chicken is in a transitional stage, both with development and with behavioral response to sympathomimetic amines. Conceivably the observed responses to CPA (i.e., subdued behavior with elevated dopamine concentrations) may be the end results of subsequent norepinephrine levels. It is not known whether CPA and/or its metabolites could inhibit catechol-*O*-methyltransferase (COMT), monoamine oxidase (MAO), and/or aldehyde dehydrogenase (ADH), thereby increasing DA concentrations. This would allow NE levels to elevate from excess DA and at the same time elevate 5HT levels producing the observed subdued behavior in chickens. The above is supported by Nishie *et al.* (11), who observed significant increases in both NE (hindbrain) and HVA (forebrain and hindbrain) concentrations 24 hr after CPA administration in mice. The reduced spontaneous motor activities parallel elevated DA and 5HT levels. Similar hypokinetic activities were reported for equipotent doses of chlorpromazine and reserpine and the neurochemical effects of CPA (at least in mice) more closely approached that of chlorpromazine.

The elevation of the acidic metabolite of DA (i.e., DOPAC) and 5HT (i.e., 5HIAA) may be either the end results of increased concentrations of the neurotransmitters or the inhibition of the transport mechanism by which these compounds are eliminated from the brain (22). CPA's ability to complex with trace metals has been suggested as the mechanism by which toxicity may occur (23, 24).

The differences in brain weight among the treatments (Table II) do not vary significantly as compared to their controls; however, the brain weight–body weight ratio (Table III) shows a significant ($P \leq 0.01$) increase at 24 hr with the 10.0 mg/kg dosed birds. This increase in the brain weight–body weight ratio is significant ($P \leq 0.01$) at 48 hr

in both the 5.0 and the 10.0 mg/kg treatment groups and in the 10.0 mg/kg treatment group at 96 hr after CPA administration. Similar observations (i.e., increases in organ weight—kidney, pancreas, and liver) have been reported as a function of CPA toxicity (25).

Poor feed conversion and decreased feed intake have been dose-related in chronic feeding studies (15, 23, 25) with CPA in chickens. Whether there is a relation between decreased feed intake (or poor feed conversion) and neurotransmitter–metabolite concentrations in brain tissue is a subject that may be addressed in future experiments. However, there are no significant differences in weight and/or changes in weight at 24, 48, and 96 hr after a single (po) dose (0.5 or 5.0 mg/kg) with CPA in this study (9). Therefore, it is doubtful that reduced feed intake occurred (except possibly at the toxic dose level, i.e., 10 mg/kg). Then too there are no significant differences among the treatments in neurotransmitter–metabolite levels at 96 hr (Table I); thus it would appear the differences observed in these levels (as compared to the controls) are the end results of CPA toxicity and not the results of reduced feed intake.

Although CPA toxicity shows variations with species and sex within a species, these investigations were designed to parallel more closely current practices in broiler operations in which 6- to 8-week-old birds are unsexed prior to marketing (26). Since CPA accumulates in edible meat, birds fed contaminated feed and not showing behavioral signs of CPA toxicity could be inadvertently marketed for human consumption. Thus the potential human toxicity of CPA needs to be assessed.

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