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Effects of Drugs That Modify Brain Monoamine Concentrations on Photoperiodically-Induced Testicular Growth in Coturnix Quail (Coturnix coturnix japonica)[1]

M. E. EL HALAWANI, W. H. BURKE and L. A. OGREN

Department of Animal Science,
University of Minnesota,
St. Paul, Minnesota 55108

ABSTRACT

The monoamine oxidase inhibitor tranylcypromine (TCP) increases brain dopamine (DA), norepinephrine (NE) and serotonin (5-HT) levels and inhibits photoperiodically-induced testicular growth in quail. Blockade of catecholamine synthesis by α-methyl-tyrosine (MT) in TCP treated quail preferentially elevates 5-HT levels and does not interfere with TCP inhibition of the gonads, however, treatment with parachlorophenylalanine (PCPA) reduces 5-HT and DA and antagonizes the action of TCP. Treatment with PCPA alone causes further augmentation of testicular response to photostimulation and reduces 5-HT and DA levels in the brain, whereas 5-hydroxytryptophan (5-HTP) administration elevates central 5-HT stores and partially inhibits testicular development.

The results are consistent with the view that 5-HT may have an inhibitory effect on the process of gonadal development; in addition, an inhibitory influence of DA may also be involved.

INTRODUCTION

Monoaminergic participation in the control of gonadal development during exposure to stimulatory photoperiods has been shown in avian species. Recent investigations demonstrated an increase in central norepinephrine (NE) turnover after photostimulation (Campbell and Wolfson, 1974; El Halawani and Burke, 1976), and pharmacological agents that interfere with NE metabolism reduce the gonadal response to light stimulation (Assenmacher and Boissin, 1972; Davies and Follett, 1974; El Halawani and Burke, 1975). These investigations favor the view that a noradrenergic mechanism participates in the normal development of the gonads in Aves. On the other hand, data have been presented which suggest that central dopamine (DA) acts to inhibit gonadal growth (El Halawani and Burke, 1975). The indolamine, serotonin (5-hydroxytryptamine; 5-HT) has also been proposed to have an antagonistic role (Calas, 1975).

Previous experiments revealed that quail maintained on a short day length (6L:18D) have quiescent testes and high brain monoamine levels when sacrificed in the dark phase of the photoperiod (El Halawani and Burke, 1975). This observation prompted the present investigation designed to determine the effects of increasing central monoamine stores on testicular development of quail.

The objectives of the present investigation were: 1) to determine whether the inhibition of monoamine oxidase might interfere with testicular development which follows exposure to stimulatory lighting schedule; 2) and having shown this, to ascertain whether a catecholaminergic or an indolaminergic mechanism was specifically concerned.

MATERIALS AND METHODS

Male quail were reared under continuous illumination for the first 3–4 weeks of life. The short "nonstimulatory" photoperiod (6L:18D) was then given for 4 weeks. When the birds were 7–8 weeks of age they were weighed and groups with equal body weights were constructed, and placed under a light regimen of 14 h of light and 10 h of darkness (0900–2300 h) daily. Feed and water were available *ad libitum*.

Beginning on the first day of photostimulation the birds were injected twice daily (0900 and 1600 h) with pharmacological agents designed to modify brain monoamine levels. Various experimental groups of quail were tested as follows: synthesis of catecholamines (CAs) was blocked with α-methyl-p-tyrosine methyl ester (MT, 250 mg/kg, i.p.) (Spector et al., 1965); synthesis of 5-HT was blocked by parachlorophenylalanine methyl ester (PCPA, 100 mg/kg, i.p.)

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(Koe and Weissmann, 1966); a precursor of 5-HT (5-hydroxytryptophan, 5-HTP, 75 mg/kg, i.p.) was injected to increase 5-HT levels (McGeer et al., 1963); synthetic luteinizing hormone-releasing hormone (LHRH, 1.25 μg/bird, i.m.) was injected to bypass the impaired CA metabolism; and degradation of monoamines was also blocked in quail treated with the aforementioned drugs. Tranylcypromine sulfate (TCP, a monoamine oxidase inhibitor, 25 mg/kg, i.p.) was utilized for this purpose. Group arrangements and the number of birds for each experiment are given in Tables 1–3.

Seven days after light stimulation and the beginning of drug treatment, the birds from each treatment were randomly killed by decapitation between 1900–2000 h. Body weights and testes weights were obtained and the whole brains were taken for determination of monoamines. Brain amines were extracted as previously described (Barchas et al., 1972). DA, NE and epinephrine (E) and 5-HT were measured by the spectrofluorometric methods of Anton and Sayre (1964), Anton and Sayre (1962) and Bogdanski et al. (1956), respectively.

The data were statistically analyzed using analysis of variance and significant ($P \leq 0.05$) differences between treatments were determined using Student-Newman-Keul's test (Snedecor and Cochran, 1967).

RESULTS

Experiment 1

Effects of depressed and elevated brain monoamine levels on testicular response to photostimulation (Table 1).

In an attempt to study the relationship between increased brain monoamines and the testicular response to light stimulation, TCP was administered on the first day of photostimulation and the following 6 days. This treatment produced a 38% increase in DA, a 50% increase in NE and a 68% increase in 5-HT, relative to the saline injected group. There was no change in E stores. The TCP-induced increase of monoamines was associated with a significant suppression of testicular development following photostimulation.

The administration of MT also produced a significant inhibition of testicular response to light stimulation, however it induced a marked decrease of DA (91%), NE (78%) and E (59%) without changing 5-HT stores. These data support earlier results from our laboratory (El Halawani and Burke, 1975). Combined treatment with TCP and MT caused further testicular inhibition from MT treatment alone. DA and NE remained depleted in this case but the 5-HT level was increased (compare groups 3 and 6).

The possibility that the decrease of testicular

TABLE 1. Effects of MT and LHRH on TCP-induced inhibition of testicular response to photostimulation of Coturnix quail.

Group	Treatment	Testis wt. (mg)	Monoamines (ng/g) DA	NE	E	5-HT
1	Saline	186.6 ± 4.5[a]	388 ± 18[a]	554 ± 40[a]	119 ± 2[a]	736 ± 40[a]
2	TCP	43.7 ± 7.2[bd]	534 ± 21[b]	830 ± 41[b]	104 ± 3[a]	1238 ± 59[b]
3	MT	57.4 ± 5.5[b]	37 ± 6[c]	119 ± 13[c]	49 ± 3[b]	784 ± 31[a]
4	LHRH	183.8 ± 16.5[a]	349 ± 29[a]	467 ± 30[a]	119 ± 3[a]	757 ± 28[a]
5	TCP + LHRH	92.4 ± 7.4[c]	517 ± 24[b]	795 ± 43[b]	124 ± 6[a]	1093 ± 69[c]
6	MT + TCP	31.8 ± 7.9[d]	79 ± 5[c]	208 ± 26[c]	75 ± 10[b]	1067 ± 41[c]

Results are expressed as means ± S.E. of 6–8 birds/group. Means within a column with different superscripts are different at the 5% level of probability.

development observed in photostimulated quail after TCP administration resulted from a peripheral effect of the drug and interference with testicular competence was tested by giving LHRH concomitantly with TCP. LHRH, when injected into TCP treated birds, partially reversed the blocking effect of TCP on the testes even though brain monoamine levels remained elevated. By contrast, daily administration of LHRH alone did not modify testicular response to photostimulation as compared to saline controls.

Experiment 2

Effects of depressed brain DA and 5-HT levels and elevated NE level on testicular response to photostimulation (Table 2).

In experiment 1, a combination of TCP and MT significantly reduced testicular development following photostimulation. Since 5-HT levels remained similar to those of quail treated with TCP alone, the inhibited testicular development resulting from TCP may have been related to the high 5-HT levels rather than the high CA stores. To test this, quail treated with TCP were also treated with PCPA.

The effects of TCP on brain monoamines were essentially identical to those of experiment 1. Increased levels relative to saline controls were 18% for DA, 40% for NE and 52% for 5-HT with no change in E levels. As in experiment 1, this was associated with a significant suppression of gonadal growth. In contrast, PCPA treatment resulted in a doubling of testicular weight and depressed DA and 5-HT levels. The suppression of testicular growth by treatment with TCP was much less marked in PCPA and TCP treated quail (compare groups 2 and 4), however, a significant level was not achieved. The combined treatment with PCPA and TCP decreased DA and 5-HT levels while it increased NE stores, relative to saline treated controls. The individual testis weights in these 2 groups showed little overlap and the means were considerably different. Only 2 males in the PCPA + TCP group fell into the same weight range as the TCP treatment alone. The failure for these means to be statistically significantly different is probably related to the high variability within the PCPA + TCP group and its influence on the overall error mean square.

Experiment 3

Effects of DA and 5-HT depletion or DA

TABLE 2. Effects of PCPA on TCP-induced inhibition of testicular response to photostimulation of Coturnix quail.

Group	Treatment	Testis wt. (mg)	Monoamines (ng/g) DA	NE	E	5-HT
1	Saline	131.2 ± 14.9^a	597 ± 23^a	451 ± 21^a	132 ± 4^a	792 ± 65^a
2	TCP	30.9 ± 4.9^b	702 ± 30^b	632 ± 15^b	131 ± 3^a	1204 ± 78^b
3	PCPA	260.3 ± 67.8^c	367 ± 16^c	413 ± 17^a	111 ± 2^a	158 ± 16^c
4	PCPA + TCP	66.9 ± 4.9^b	498 ± 25^d	619 ± 34^b	121 ± 2^a	249 ± 12^c

Results are expressed as means ± S.E. of 8 birds/group. Means within a column with different superscripts are different at the 5% level of probability.

depletion alone on testicular response to photostimulation (Table 3).

In experiment 2, the tryptophan hydroxylase inhibitor PCPA caused not only a depletion of 5-HT but also decreased DA levels, confounding any distinction between the involvement of 5-HT and DA in testicular growth. Therefore, this experiment was designed to increase 5-HT levels while holding DA constant and to study the effect of increased 5-HT levels on testicular weight.

As in experiment 2, PCPA significantly depressed 5-HT and DA levels, however, NE and E levels were unchanged. Again as in experiment 2, this was accompanied by an augmentation of light-induced testicular development. Treatment with 5-HTP increased 5-HT (40%) without affecting CA levels, and produced a marked inhibition of testicular response to photostimulation (compare groups 1 and 3). Combined treatment with PCPA and 5-HTP elevated 5-HT and depressed DA levels. The testicular development in quail injected with PCPA and 5-HTP was comparable to that of saline treated controls.

Experiment 4

Effects of limited feeding on testicular response to photostimulation.

Treatment with TCP resulted in body weight suppression. Although the reduction in body weight was not significant, the possibility was considered that the decreased testicular development found in TCP treated quail was merely due to the reduced food intake, with consequent loss of body weight resulting from the administration of the drug. Testes weights were measured in photostimulated quail whose feed intake was limited to that consumed by TCP-treated quail.

This experiment shows that despite a 6 g decrease in body weight, no significant decrease in testicular response to photostimulation was present in feed restricted quail. Monoamine levels were essentially unchanged in the brains of limited fed quail as compared to full fed controls, with the exception of E. The mean E level in the full fed control group was higher than usual and both the TCP and limited feed birds were significantly lower than it.

TABLE 3. Effects of PCPA and/or 5-HTP on the testicular response to photostimulation.

Group	Treatment	Testis wt. (mg)	Monoamines (ng/g)			
			DA	NE	E	5-HT
1	Saline	149.1 ± 19.7[a]	429 ± 36[a]	576 ± 22[a]	138 ± 4[a]	663 ± 33[a]
2	PCPA	200.4 ± 8.3[b]	283 ± 31[b]	545 ± 24[a]	130 ± 3[a]	277 ± 31[b]
3	5-HTP	80.9 ± 6.9[c]	431 ± 24[a]	571 ± 19[a]	133 ± 2[a]	932 ± 48[c]
4	PCPA + 5-HTP	137.8 ± 18.5[a]	245 ± 19[b]	539 ± 28[a]	132 ± 2[a]	899 ± 56[c]

Results are expressed as means ± S.E. of 8 birds/group. Means within a column with different superscripts are different at 5% level of probability.

DISCUSSION

Results of the present investigations support the view that high brain monoamine levels

inhibit testicular development of quail in response to photostimulation. Augmentation of 18%–38% for DA, 40%–50% NE and 52%–68% 5-HT were associated with a 77% deficit in testicular growth. The inhibitory effect of high monoamine levels as a consequence of monoamine oxidase inhibition on gonadal function corroborate other data with mammalian species (Kordon et al., 1968).

There is reason to believe that this reduced testicular growth is not due to an inability of the pituitary to release gonadotropin and/or a result of toxic effects of the drugs, since treatment with LHRH of TCP-treated quail partially restored testicular growth (Table 1). The testes of quail held on a nonstimulatory photoperiod are not directly responsive to LHRH. Thus, in the present studies, the pituitary was competent to release gonadotropins in the face of the elevated biogenic amines and the testes were competent to respond. Moreover, limited feeding resulting in a slight decrease in body weight in TCP-treated quail cannot account for the reduction in testicular response to photostimulation. Thus, from these and previous data (Calas, 1975; Davies and Follett, 1974; El Halawani and Burke, 1975), it appears that brain biogenic amines play a role(s) in photoperiodically-induced testicular development in quail.

In these studies whole brain levels of monoamines were determined. In view of the heterogeneity and the functional complexity of the brain, there is no simple way to ascertain which amine is specifically concerned with testicular development following photostimulation. If the changes in whole brain monoamines also represent the changes at the level of neural structures involved in photoperiodism, it is then valid to speculate on their roles in regulating reproductive function.

Preferential elevation of 5-HT stores, as a consequence of the action of TCP given concomitantly with MT did not prevent the blocking effect of the former on testicular growth (Table 1, group 6). The possibility was considered that depletion of CAs rather than an increase in 5-HT stores was responsible for the testicular inhibition. This supposition is not supported if one considers that combined treatment with TCP and PCPA reduced 5-HT stores and partially prevented the blocking effect of the latter on testicular response to photostimulation (Table 2; compare groups 2 and 4). Moreover, combined treatment with TCP and MT augmented brain 5-HT levels and produced further suppression of testicular growth than MT treatment alone (Table 1; compare groups 3 and 6). Brain 5-HT participation also appears likely considering the depression in testicular development of quail in which brain levels of the indolamine were augmented as a consequence of 5-HTP administration (Table 3). Taken together, these findings suggest that the TCP-induced testicular inhibition is mediated by an accumulation of 5-HT and serotonergic mechanisms can inhibit gonadal development. This view agrees with the observations of Calas (1975), that treatment of quail with 5, 7-dihydroxytryptamine caused a degeneration of serotonergic fibers, and augmented the testicular response to photostimulation.

The tryptophan hydroxylase inhibitor, PCPA, augmented the testicular response to photostimulation, and reduced both DA and 5-HT levels. Thus, the gonadal augmentation could have been produced by depletion of one or both of these monoamines. That increasing 5-HT stores in the presence of normal DA levels diminishes the photoperiodic response of the gonads (Table 3, group 3) whereas, DA depletion prevents 5-HT accumulation-induced testicular inhibition (Table 3, group 4) implies that testicular inhibition does not rest solely on 5-HT accumulation. Therefore, an inhibitory effect of a dopaminergic component in regulating testicular development cannot be excluded. Needless to say, the interactions between DA and 5-HT mechanisms in the brain need to be further investigated. Earlier data (El Halawani and Burke, 1976) have shown that during periods of ovarian regression in female turkeys, brain DA turnover is high and these workers found that during incubation when ovaries are fully regressed 5-HT turnover is increased. It was found that L-DOPA increased brain DA levels with no change in NE, E or 5-HT content and resulted in testicular growth depression (El Halawani and Burke, 1975).

As already mentioned, it has been indicated from various studies that an active noradrenergic mechanism is essential for transforming the photoperiodic information necessary for testicular development (Campbell and Wolfson, 1974; Davies and Follett, 1974; El Halawani and Burke, 1975). This is in contrast to the apparent restraining effect of 5-HT and/or DA. Whether these mechanisms represent a dual central control for gonadal response to photostimulation remains an open question.

Presumably the changes in gonadal growth which accompany the changes in brain amines are associated with changes in gonadotropin secretion. Unpublished observations from this laboratory show that changes in LH levels closely paralleled changes in testes weight in the study of El Halawani and Burke (1975). The findings of Davies and Follett (1974) show that 6-hydroxydopamine decreases plasma LH levels in photostimulated quail. However, changes in prolactin secretion associated with brain monoamine manipulation cannot be ruled out and may explain the different effects of PCPA and LHRH on gonadal development. Dopamine may increase prolactin secretion as indicated by the increase in plasma prolactin in cockerels treated with CB154 (2-Br-α-ergocryptine-methanesulphonate), a DA receptor stimulating compound (Scanes et al., 1976). Camper and Burke (1977) have shown that prolactin, presumably through a direct action on the gonad, inhibits FSH and LH-induced increases in serum steroid levels. It is therefore reasonable to assume that PCPA treatment which reduced brain DA levels (Table 2, group 3 and Table 3, group 2) may have interfered with prolactin secretion, thus explaining the augmentation of testicular growth observed in PCPA-treated birds.

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