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Antifertility effects of clonidine in laying hens

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Summary. Clonidine was anovulatory and markedly antigonadal in laying hens when infused for 1 week from minipump implants at daily rates of 1.08 mg per hen or greater. The ovaries of hens treated with clonidine responded to FSH injections which suggests that the antigonadal effect of clonidine resulted from a reduction in the output of gonadotropin by the pituitary. These data suggest that α_2 receptors may be important in regulating avian fertility.

There are several indications that adrenergic systems play an important role in regulating avian fertility. For example, the α-adrenergic blocking agents dibenamine[1] and phenoxybenzamine[2,3] were anovulatory in laying hens, and the catecholamine depleting agent reserpine reduced luteinizing-hormone concentrations in the plasma of cockerels as well as laying hens[4]. For *Coturnix* quail, the testicular development induced by lengthening daylight was blocked by inhibiting either tyrosine hydroxylase, the branch enzyme of catecholamine synthesis, or dopamine β-hydroxylase, whose product is norepinephrine[5]. A recent publication from this laboratory demonstrated that several inhibitors of dopamine β-hydroxylase had potent antigonadal and anovulatory activity in laying hens[6]. Since the anovulatory effect could be overcome by a single injection of luteinizing hormone releasing hormone, the hydroxylase inhibitors appeared to block fertility within the hypothalamus or elsewhere within the central nervous system. Taken together, these publications suggest that central α-adrenergic activity is required for avian fertility.

The introduction of highly specific α-adrenergic agonists and antagonists has resulted in the recent division of α-adrenergic receptors into 2 subclasses - α_1 and α_2[7]. α_1-Receptors, which always appear to be located postjunctionally, have relatively high affinity for the agonist methoxamine, while α_2-receptors, which can be located either prejunctionally or postjunctionally, have a relatively high affinity for the agonist clonidine. The major role of α_2-receptors appears to be the reduction of sympathetic outflow. Since phenoxybenzamine is a relatively specific antagonist for α_1-receptors, the work quoted above suggests that α_1-receptors play a role in avian reproduction. It was of

Table 1. Egg production and reproductive systems of hens treated with clonidine for 1 week

	Body weight change (percent of initial weight ± SD)	Ovarian wt ± SD (g)	Oviduct wt ± SD (g)	Percent of hens laying eggs on day 1	2	3	4	5	6	7
A. Oral treatment (fed)[a]										
Dose (ppm)										
0	+0±2	51±3	56±5	83	67	83	100	100	100	50
100	+5±3*	44±23	50±19	50	100	100	0	75	50	50
200	+6±4*	26±23*	37±17*	100	50	75	0	100	50	0
400	+5±4*	16±4*	22±3*	75	50	50	25	25	25	0
600	+2±2	11±5*	19±6*	50	75	75	25	25	0	0
B. Parenteral treatment (implant)[b]										
Daily dose (mg/hen)										
0	−1±2	58±10	61±9	100	100	80	60	60	100	80
0.48	+4±1*	51±9	67±10	100	100	67	100	67	100	67
1.08	+1±8	13±6*	23±5*	67	33	33	0	0	0	0
2.16	+5±5*	9±3*	23±5*	100	100	0	0	0	0	0

*Significantly different from untreated (t-test: $p < 0.05$). [a]Each treated group was of 4 hens and the untreated group was of 6 hens. [b]Each treated group was of 3 hens and the untreated group was of 5 hens.

Table 2. Reversal of clonidine's antigonadal effect by FSH[a]

Clonidine in diet (ppm)	Daily FSH (mg)	Ovarian wt ± SD (g)	Oviduct wt ± SD (g)	Percent of hens laying eggs on day 1	2	3	4	5	6	7
0	0	50 ± 3	65 ± 2	66	100	33	100	100	100	100
	0.13	72 ± 19	65 ± 4	100	66	100	100	100	100	66
	0.66	162 ± 19	74 ± 7	100	66	66	100	0	100	0
	3.33	179 ± 41	60 ± 10	66	66	33	33	33	0	33
600	0	15 ± 6*	23 ± 4*	66	0	33	33	66	0	0
	0.13	24 ± 13*	31 ± 5*	100	66	0	33	33	0	0
	0.66	123 ± 23	56 ± 9	33	66	66	100	0	33	0
	3.33	184 ± 31	61 ± 11	66	33	0	0	33	0	33

[a]3 hens received each treatment. *Significantly different from those hens receiving the same dose of FSH but not treated with clonidine (t-test: $p < 0.05$).

interest to determine if α_2-receptors might also be involved and to this end we have investigated the effect of the prototypic α_2-agonist clonidine on avian reproduction.

Materials and methods. Clonidine was synthesized[8] and FSH obtained from Calbiochem (San Diego, CA). Amounts of FSH are expressed as mg of the Armour Standard based on equivalence information from the supplier.

To determine parenteral activity, clonidine, as the free base, was suspended in 0.2 M phosphate buffer, pH 7.2 at 20, 45, or 90 mg/ml. The pH was returned to about 7 with concentrated HCl. The solutions were then passed through a 0.45 µm filter and used to fill Alzet Osmotic Minipumps, Model 1701 (Alza Corporation, Palo Alto, CA). The hens were anesthetized locally with 2% lidocaine HCl and the pumps were implanted s.c., dorsally at the base of the neck, via a small incision which was closed with a wound clip. The test was then conducted as described[6]. Daily dose was calculated from the delivery rate of the pumps which was stated to be 1 µl per h by the manufacturer. All other procedures were as described previously[6].

Results and discussion. Clonidine, at 200, 400 or 600 ppm in the feed of laying hens depressed egg production and significantly decreased ovarian and oviduct weights after 1 week of treatment (table 1, part A). The decreased ovarian weight was due to atresia of virtually all mature follicles without growth of new follicles. Oviduct regression presumably resulted from the inability of the atretic follicles to supply estrogenic support. These effects closely resemble those of the dopamine hydroxylase inhibitors[6]. Body weight changes were positive for the treated hens.

To determine parenteral antifertility efficacy, solutions of clonidine in minipumps were implanted for a 7-day period at the conclusion of which the reproductive systems were examined. The results in table 1, part B, indicate that hens receiving doses of 1.08 mg or more showed the same severe atrophy of ovaries and oviducts as those treated with 400 or 600 ppm in the diet. Since the average daily food consumption of these hens is about 100 g, 400 ppm in the diet corresponded to an average daily dose of 40 mg, compared with the effective parenteral dose of 1 mg. This discrepancy in effective doses suggests that clonidine is not absorbed efficiently from the avian intestinal tract.

In the experiment of table 2, hens treated with 600 ppm clonidine in the diet for 1 week received daily injections of porcine follicle stimulating hormone (FSH). Hens receiving 0.66 or 3.33 mg FSH daily experienced comparable ovarian hypertrophy whether or not they were treated with clonidine. This indicates that the ovaries of hens treated with clonidine were capable of responding to FSH and suggests that the antigonadal effect of clonidine was not due to a block at the ovarian level but resulted instead from a reduction in gonadotropin output by the pituitary. Hens receiving the lower dose of FSH and treated with clonidine had significantly smaller ovaries than those receiving the same doses of FSH and not treated with clonidine, presumably because ovarian weight reflected the sum of exogenous FSH plus endogenous gonadotropins and the latter were reduced in hens treated with clonidine. Although the data in table 2 suggest strongly that the antigonadal effect of clonidine is due to a reduction in gonadotropin output by the pituitary, verification of this proposal will require direct measurement of gonadotropins in the plasma of hens treated with clonidine.

It should also be noted that the potent antifertility effects of clonidine in hens, although suggestive, do not provide conclusive proof that α_2-receptors regulate avian fertility, especially in light of reports that clonidine may also interact with α_1-adrenergic[7] and H_2-histaminergic[9], as well as purinergic systems[10]. One way of addressing this problem would be to compare the antifertility activities of clonidine and various clonidine derivatives with their affinities for the avian α_2-receptor, which could be determined in vitro by binding assays similar to those published[11]. Alternatively, antifertility efficacies could be compared with avian hypotensive efficacies since hypotension is the archetypal α_2-mediated effect of clonidine[8]. Correlation among these biological effects would strengthen the proposal that the avian antifertility activity of clonidine is mediated by an α_2-receptor interaction. Experiments along these lines are in progress.

In contrast to avian species, mammals do not appear to exhibit antifertility effects when treated with clonidine. When infused into human males at levels sufficient to sedate and to elevate plasma growth hormone, clonidine had no effect on plasma FSH or LH[12]. Nor were the serum levels of either gonadotropin affected when it was implanted into the amygdala of rats[13]. The marked contrast between clonidine's potent avian antifertility action and its apparent lack of antifertility action in mammals suggests major differences in the neuroendocrine regulation of reproduction between birds and mammals.

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Characteristic responses to L-dopa of cerebral blood flow and EEG pattern in stroke-prone spontaneously hypertensive rats[1]

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Summary. L-Dopa given i.p. increased regional cerebral blood flow and influenced EEG patterns in stroke-prone spontaneously hypertensive, but not in normotensive rats. The feasibility of this approach for determining the pathology of the cerebral vessels and the blood-brain barrier warrants further study.

There are little data on the effect of L-dopa on cerebral blood flow in laboratory animals[3], and apparently none regarding the effect of this norepinephrine precursor on the pathophysiological state of cerebral circulation. We examined the effects of L-dopa on cerebral blood flow (CBF) and cortical electrical activity in spontaneously hypertensive stroke-prone and stroke-resistant and normotensive rats.

Male spontaneously hypertensive stroke-prone (SHR-SP), stroke-resistant (SHR-SR) and Wistar-Kyoto (WK) rats, 6 months old, were used. The experimental groups included 8–7 animals. For the regional cerebral blood flow (rCBF) measurements, rats were prepared according to the method described by Yamori and Horie[4]. Under sodium pentobarbital anesthesia (40 mg/kg i.p.) platinum electrodes were bilaterally implanted into the frontal cerebral cortex. rCBF was measured in conscious unrestrained rats by the hydrogen clearance method[5]. In each rat rCBF was first measured 2 weeks after the implantation of electrodes. All rats implanted with hyposensitive electrodes and with outlying values of rCBF were excluded from further analysis. The same set-up as for rCBF measurements was used for cortical EEG registration[6,7]. Blood pressure was measured in conscious rats by the tail plethysmographic method.

Experimental procedure was as follows: after control rCBF measurements L-dopa ('Nakarai', 100 mg/kg dissolved in physiological saline at a temperature of 45 °C) was given i.p. in the form of a fine-grained suspension. The measurements were performed 0.5, 1, 1.5, 2, 3, 3.5 and 4 h thereafter. Cortical EEG was recorded in the same animals during the intervals of rCBF measurements and the records were analyzed according to the methods of Yamori et al.[6,7]. The cortical EEG was recorded from 20 min up to 4 h after L-dopa injection. Additionally, the state of the blood-brain barrier was assessed in 2 SHR-SP rats by determining the effect of Penicillin G on the EEG[8]. A similar group of animals was used to measure the arterial blood pressure. Student's t-test was used for statistical analysis.

Two different types of rCBF response were observed 0.5 h after i.p. administration of L-dopa (table). In SHR-SP which showed an obvious reduction in rCBF before L-dopa administration, as described previously[4], the significant increase observed in rCBF remained at these elevated levels for over 2.5 h. In contrast, in SHR-SR and WK there was a decrease in rCBF following L-dopa administration and a stepwise recovery occurred within 4 h. The administered dose of L-dopa had no effect on the blood pressure in SHR-SP and SHR-SR. A significant increase in blood pressure was noted only in WK 30 min and 1 h after L-dopa administration.

After the pretreatment with peripheral decarboxylase inhibitor MK-486, L-dopa resulted in a marked rise in rCBF by 39% in SHR-SP and an increase in flow by 11.5% in WK rats.

Differences in the EEG pattern could be observed in SHR-SP depending on whether they were awake or drowsy[6,7]. Control EEG recordings during the waking state in SHR-SP were similar to those observed in the other groups and there was a predominance of 20–50 cps waves (amplitude 10–150 μV). A predominance of θ waves superimposed by β waves was observed in control SHR-SP in a drowsy state. These θ waves were in the form of diffused spindles over 5–15 sec (fig.). After the L-dopa administration the θ waves appeared only in SHR-SP. From 20 min up to about 1 h after L-dopa administration, a further increase of the amplitude (to 600 μV) and the number of θ waves were seen. Also high voltage (400 μV) δ waves often appeared. L-Dopa administration was without apparent effect upon the EEG pattern in both SHR-SR and WK. Administration of Penicillin G to SHR-SP rats did not alter the EEG pattern, indicating that the function of the blood-brain barrier was not extensively impaired[8].

These results were obtained after L-dopa had been given i.p. in a dose which in WK rats only produced an increase in blood pressure due to the convention to L-dopa to norepinephrine in the peripheral vessels. In SHR-SP and SHR-SR such peripheral pressor effects seemed to be cancelled by the central depressor effect[9], resulting in no pressor response after L-dopa administration. In this experiment L-dopa was given i.p in order to examine rCBF, in conscious, unrestricted animals. After this administration there was no enhancement in the respiratory frequency which could, in certain instances, result in hyperventilation. Our observations do not elucidate the mechanism of L-dopa action on CBF; however, the marked effect on both cerebral flow and the electrical activity of the brain mainly observed in the SHR-SP with severe hypertension could be the result of enhancement of the amount of L-dopa available at the blood-brain interface and/or increased penetration of exogenous L-dopa into the central dopaminergic