The Effect of Reserpine on Adrenal Medulla of the Pigeon—a Cytochemical Investigation

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Introduction

In recent years, reserpine has been used extensively in order to have a better understanding of some specific cytophysiological problems associated with the mammalian adrenal medulla. Thus, Holzbauer and Vogt (1956) found that this alkaloid releases adrenaline and noradrenaline from the adrenal medulla of the cat. In the rabbit, the reserpine causes complete adrenaline depletion (Carlsson and Hillarp 1956). Relatively smaller dosage of reserpine brings out complete noradrenaline depletion in the rat (Camanni *et al.* 1958, Eränkö and Hopsu 1958) and the hamster (Camanni and Molinatti 1958). In mouse adrenal, however, Eränkö and Hospu (1958) noted a parallel loss of both catechol hormones. A marked difference in sensitivity to reserpine in rats of different strains has been noted by Coupland (1958). Repeated subcutaneous injections of the Rauwolfia alkaloid deplete 50% of the catechol amine from rat medulla and it takes about 21 days for complete resynthesis (Callingham and Mann 1958). On the other hand, it has been found by Coupland (1959a) that complete hormonal resynthesis requires 14 days in total, as against 21 days after a reserpine-induced complete depletion of catechol amines. From his skillful grafting experiments, this author (Coupland 1959b) has proved that nervous mediation is not necessary during the period of resynthesis.

From the work cited above it is evident that the functional response of the adrenal medulla to reserpine administration is very much variable. However, the basic problem interpolated with these studies is to know the exact mechanism of reserpine action on the chromaffin cells. Though the proposition is mostly an unsolved one, yet its exploration is certainly very important. To arrive at a more or less unified and generalized concept on the mode of reserpine (and other tranquillizers) action through amine humoral systems, a comparative endocrinological approach seems desirable. In this view, the present authors have used the pigeon as their experimental material to study the effect of Rauwolfia alkaloid on its adrenal medulla. It may be recalled here that all the previous observations on the topic concerned was based upon animal experiments involving only a particular group of mammals i.e.,

rodents. Further it may also be noted that the species so far studied contain adrenaline as the preponderant hormone in their medullary tissue. The pigeon deviates from these species in having more noradrenaline concentration in its adrenal (Ray and Ghosh 1961 and Ghosh and Ghosh 1962).

In the present report an effort has been made to study the distribution and concentration of cytochemically demonstrable adrenaline and noradrenaline in the adrenal medulla of the normal pigeons, and to determine the catechol hormone responsiveness to reserpine treatments.

Material and methods

Adult pigeons (average weight range 250–300 gm) were selected as the experimental material. The birds were injected intramuscularly with different dosage of reserpine ("Serpasil"—Ciba laboratories) varying from 0.05 mg–3 mg per 100 gm of body weight. Before the commencement of injections, birds were kept at least for seven days in a normal husbandry condition. After the injections 24 hours were allowed to lapse and then the treated as well as control pigeons were anesthetized, killed and their adrenals were quickly dissected out and transferred to suitable fixatives.

For total catechol amines, adrenal of one side was treated with a mixture of 5% potassium dichromate and 5% potassium chromate (10 : 1) solutions for 48 hours and tissues thus treated were transferred to 10% formalin for 24 hours (Hillarp and Hökfelt 1955). To demonstrate noradrenaline, the glands of the other side were treated with saturated solution of potassium iodate for 24 hours and subsequently these were immersed in 10% formalin for 48 hours (Hillarp and Hökfelt 1955). Followed by these treatments 30 μ paraffin sections were made after embedding in a mixture of Beeswax, ceresin and paraffin.

Results

Control

Chromate-dichromate reaction. All medullary strands show the presence of fairly large brown coloured particles associated with a yellowish brown to yellow coloured homogeneous cellular matrix. The peripheral region of the individual medullary strand in most cases reveals the reaction of maximum intensity, whereas the central portion presents a material of rather weak stainability (Fig. 1).

Iodate reaction. The responsiveness to this reaction is indicated by the presence of brown granules of various size and shape (Fig. 2). In most of the strands the reactive materials are present only at the peripheral region and in the remaining — the positive substances are distributed more or less in a uniform manner (Table 1). The total area of medullary tissue occupied by iodate positive materials is about 57% (Ghosh and Ghosh 1962).

Figs. 1-6. Photomicrographs of sections through the adrenal gland of *Columba livia*. 1, all medullary strands of a normal bird show a deeply positive chromate-dichromate reaction. Intensity is slightly less at the central region. $\times 250$. 2, iodate reaction in the control pigeon showing its granular nature. $\times 250$. 3, medulla showing significant loss of catechol amine mostly from the central region after treatment with reserpine (.05 mg/100 gm). $\times 250$. 4, almost negative iodate reaction in medulla is detectable after the above dosage of treatment. $\times 250$. 5, positive chromate-dichromate reaction restricted to the periphery is visible after 1.5 mg/100 gm reserpine treatment. $\times 250$. 6, a complete negative picture of chromate-dichromate reaction caused by reserpine of dosage 2 mg/100 gm. $\times 250$.

Reserpine treatment

Dosage .05 mg/100 gm bd. wt.

Chromate-dichromate. Overall decrease of positive materials has been noticed in all the medullary strands examined. This, however, is more marked in the central portion of individual chromaffin cords (Fig. 3).

Iodate. None to almost negative reaction is encountered in medullary strands of the pigeons treated with the present schedule (Fig. 4).

Dosages .10 mg, .25 mg, .50 mg/100 gm bd. wt.

Chromate-dichromate. The above mentioned dosages are not capable of altering the peripheral reaction of the medullary cords to a perceptible extent. However, a pronounced loss of reactive material (presumably adrenaline) is evident in the core of these strands.

Iodate. The schedules of treatments under consideration cause an almost total depletion of cytochemically detectable noradrenaline from the adrenomedullary patches.

Dosages .80 mg, 1.50 mg, 1.70 mg/100 gm bd. wt.

Chromate-dichromate. There is virtually a complete loss of adrenaline (?) from the core of practically all the chromaffin strands. However, the peripheral reaction in these strands is still retained to an perceptible extent (Fig. 5).

Iodate. No iodate reaction is visible in any of the medullary cord of treated birds.

Dosages 2 mg, 2.50 mg/100 gm bd. wt.

Pigeons administered with reserpine according to above mentioned schedule present absolutely no reaction in their adrenal medulla either with the chromate-dichromate (Fig. 6) or with the iodate method.

Dosage 3 mg/100 gm bd. wt.

Pigeons are unable to thrive when the reserpine dosage is raised to 3 mg per 100 gm of body weight.

Discussion

From the data presented in this report, there seems to be no doubt that treatments with reserpine significantly provoked adrenomedullary stimulation in the pigeon. With regard to the stimulatory effects of reserpine on suprarenal medulla manifested by catechol amine depletion, the results obtained here are not out of keeping with those reported for the rabbit (Carlsson and Hillarp 1956; Kroneberg and Schumann 1957), hamster (Camanni and Molinatti 1958), rat, guinea pig, and cat (Holzbauer and Vogt 1956; Molinatti *et al.* 1958). However, when the response of two principal medullary catechol amines to reserpine is considered separately in these mammalian

species a distinct differential behaviour of the catechols becomes apparent. In rats (Eränkö and Hospu 1958, Camanni *et al.* 1958) and hamsters (Camanni and Molinatti 1958), the Rauwolfia alkaloid in a rather small dosage may cause a specific depletion of noradrenaline while the adrenaline content remains unaffected. A parallel loss of both catechol amines has been noted in the cat (Holzbauer and Vogt 1956) and the mouse adrenal medulla (Eränkö and Hospu 1958) due to reserpine treatment. The present report on the pigeon indicates that a small dose (.05 mg/100 gm. bd. wt.) of reserpine may cause an almost complete depletion of noradrenaline from the suprarenal medulla. This, however, can not be considered as a case of 'specific depletion' (compare the results on rats and hamsters, *vide supra*) because the same dosage of this alkaloid is also capable of reducing the adrenaline content of medullary cords to a certain extent (*see* Table 1: Tr. I, Chr.-dichr.). It is interesting to find that with a slight increase in the dosage level (.10 mg.) — the medullary cords completely evacuate their noradrenaline content while the adrenaline

Table 1. Response of medullary catechol amines to reserpine treatment (*C. livia*)

Group*	Dosage: mg/100 gm. bd. wt. (Daily treatment)	Reaction intensities in individual medullary cord		
		Chr. -dichr.	Iodate	
Control (6)	—			**
T. I (2)	.05
Tr. II (2)	.10		Same as above	
Tr. III (1)	.25			
Tr. IV (1)	.50			
Tr. V (1)	.80		Entirely negative	
Tr. VI (2)	1.50			
Tr. VII (2)	1.70			
Tr. VIII (3)	2.00	Whole gland remains unreactive Proved to be fatal		
Tr. IX (2)	2.50			
Tr. X (2)	3.00			

* Numericals in parenthesis denote the number of birds.

** This pattern is most common.

storage in the peripheral zone of an individual cord shows a good deal of resistance. The reserpine treatment even with considerable higher dosages (.80/1.50/1.70 mg) seems to cause only a slight hormonal loss from this zone. The similar experimental conditions, however, produce a rather pronounced depletory effect on the adrenaline contents of the core in those medullary strands (Table: Tr. II-Tr. VII, Chr.-dichr.). Thus, in the pigeon, the medullary noradrenaline seems to be more sensitive to stimulation by reserpine when compared to the response of adrenaline (particularly that occupying the peripheral zone of an individual cord). The differential response elicited by the adrenaline and noradrenaline-containing cell groups of this species to the Rauwolfia alkaloid poses a fundamental problem of the neuroendocrinology which merits some comments. A joint biophysical and biochemical approach in future down to a ultracellular level — may help us for a better understanding of this intriguing problem at least to a certain extent.

It is agreed upon that the splanchnic nerve fibres innervate the medullary chromaffin cells in a direct manner and it is also fairly well known that the nerve endings are of cholinergic type (Feldberg *et al.* 1934). While studying the splanchnic nerve endings in rabbit suprarenal medulla with the aid of electron microscope, De Robertis and Ferreira (1957) have observed many vesicular components ('synaptic vesicles') in those nerve terminals. They have further noted that an increase in number of these 'synaptic vesicles' is associated with the enhanced rate of catechol amine secretion. In view of this it may be suggested that in the material under report, the nerve endings in contact with noradrenaline-containing cell groups are possibly capable of propagating 'synaptic vesicles' at a very rapid rate even with an extremely minute dosage of reserpine. In all probability this mechanism is less efficient in case of adrenaline-containing cell groups. The validity of this concept, however, is resting upon a future electron microscopic study of the adrenomedullary cords of this avian species pretreated with reserpine.

If it is accepted that medullary catechol amines are stored in granules with covering membranes (Hillarp and Nilson 1954, Blaschko *et al.* 1955), then the release of medullary hormones will certainly involve an escape from the membrane-bound compartments. In the light of these findings, the differential release of adrenaline and noradrenaline from the pigeon medulla in response to reserpine treatments appears to be suggestive. If we conceive a difference in membrane structure associated with adrenaline and noradrenaline granules — then the specific hormonal release mechanism noted in the present experiments may be partially explained.

The problem how reserpine causes medullary amine depletion in cellular level and in a species-specific manner is far from being solved (Welsh 1958, Hillarp 1960). It is hoped, with the combined efforts of the electron microscopist, molecular biochemist and the physiologist, such perplexing proposition will, in time, be clarified.

Summary

In the present report the localization and concentration of cytochemically demonstrable adrenaline and noradrenaline in the suprarenal medulla of normal and reserpine-treated pigeons have been described.

It has been found that even a very minute dose (.05 mg/100 gm b. wt.) of reserpine can cause an almost total depletion of noradrenaline. Along with this a loss of adrenaline from the core of individual cords is also noticed. It is interesting to note that considerably high dosages (.80 mg-1.70 mg) of reserpine are unable to cause depletion of adrenaline from the peripheral zone of the chromaffin cords. The differential response of adrenaline and noradrenaline to reserpine in the pigeon suprarenal medulla has been pointed out and discussed in the light of current researches.

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Literature cited

- Blaschko, H., Hagen, P. and Welch, A. D. 1955. Observations on the intracellular granules of the adrenal medulla. *J. Physiol. (London)* 129: 27-49.
- Callingham, B. A. and Mann, M. 1958. Adrenaline and noradrenaline content of the adrenal gland of the rat following depletion with reserpine. *Nature, London* 181: 423.
- Camanni, F., Losana, O. and Molinatti, G. M. 1958. Selective depletion of noradrenaline in the adrenal medulla of the rat after administration of reserpine. *Experientia* 14: 199-201.
- Camanni, F. and Molinatti, G. M. 1958. Selective depletion of noradrenaline in the adrenals of the hamster produced by reserpine. *Acta Endocrin.* 29: 369-374.
- *Carlsson, A. and Hillarp, N. Å. 1956. Release of adrenaline from the adrenal medulla of rabbits by reserpine. *Kungl. Fysiogr. Sällsk. i Lund Förhandl.* 26: Nr. 8, 1-2.
- Coupland, R. E. 1958. Strain sensitivity of albino rats to reserpine. *Nature (London)* 181: 930-931.
- 1959a. The catechol amine content of the adrenal medulla of the rat following reserpine induced depletion. *J. Endocrin.* 18: 154-161.
- 1959b. Synthesis and storage of pressor amines in adrenal medullary grafts. *J. Endocrin.* 18: 162-164.
- De Robertis, E. and Ferreira, A. V. 1957. Submicroscopic changes of the nerve endings in the adrenal medulla. *J. Biophysic. and Biochem. Cytol.* 3: 611-614.
- Eränkö, O. and Hopsu, V. 1958. Effect of reserpine on the histochemistry and content of adrenaline and noradrenaline in the adrenal medulla of the rat and mouse. *Endocrin.* 62: 15-23.
- Feldberg, W., Minz, B. and Tzudzimura, H. 1934. The mechanism of the nervous discharge of adrenaline. *J. Physiol. (London)* 81: 286-304.
- Ghosh, Ira and Ghosh, Asok. 1962. A comparative cytochemical study on the avian suprarenal medulla. *Proc. Zool. Soc., Symposium on Cell Biology* 15: 135-149.

- Hillarp, N. Å. and Hökfelt, B. 1955. Histochemical demonstration of noradrenaline and adrenaline in adrenal medulla. *J. Histochem. Cytochem.* 3: 1-5.
- and Nilson, B. 1954. The structure of the adrenaline and noradrenaline containing granules in the adrenal medullary cells with reference to the storage and release of sympathomimetic amines. *Acta Physiol. Scand.* 31: Suppl. 113, 79-107.
- Hillarp, N. Å. 1960. Effect of reserpine on the nucleotide and catecholamine content of the denervated adrenal medulla of the rat. *Nature, London* 187: 1032.
- *Holzbauer, M. and Vogt, M. 1956. *J. Neurochem.* 1: 8.
- *Kroneberg, G. and Schumann, H. J. 1957. *Arzneimittel-Forschung* 7: 279.
- *Molinatti, G. M., Camanni, F. and Losana, O. 1958. *Arch. Int. Pharmacodyn.* 116: 483.
- Ray, I. and Ghosh, A. 1961. The chemical cytology of the adrenal medulla in the domestic pigeon. *Acta Histochem.* 11: 68-77.
- Welsh, J. H. 1958. Neuroendocrine substances. In *Comparative Endocrinology*, ed. A. Gorbman (P. 221-233) John Wiley and Sons Inc., New York.

* Not seen in original.