Influence of Splanchnic Nerve on the Resynthesis of Adrenomedullary Catecholamines Post-reserpine-induced Depletion in the Pigeon, *Columba livia*

SUSHIL KUMAR MAHATA and ASOK GHOSH  
*Histophysiology Laboratory, Department of Zoology, University of Calcutta, 35, Ballygunge Circular Road, Calcutta 700019*

(Received 3 March 1986; after revision 14 April 1986)

One-day treatment of reserpine (0.8mg/100g body wt) to unilaterally denervated birds caused marked diminution of catecholamine fluorescence and nearly complete depletion of spectrofluorometrically determined norepinephrine (NE) and epinephrine (E) from the innervated and denervated adrenal medulla of the pigeon. Resynthesis of NE was completed after 6 days and exceeded the control value after 9 days while resynthesis of E was not completed even after 9 days of reserpine-induced depletion in the innervated adrenal gland. Denervation, however, markedly prevented the restitution of both NE and E in the adrenal medulla of this species. This indicates that splanchnic nerve somehow induced restitution of catecholamines in the adrenal gland. However, resynthesis of catecholamine in the denervated gland was under the influence of hormones, possibly ACTH. Further, resynthesis of NE was more in comparison to E in the denervated gland. This differential pattern of restitution of NE and E was possibly due to the selective association of intramedullary adrenergic nerve fibres with NE cells only.

Key Words: Splanchnic denervation, Catecholamines, Resynthesis, Histofluorescence, Spectrofluorometry

Introduction

Although in the biosynthesis of catecholamine, the role of splanchnic nerve is fairly well understood, particularly in the regulation of Tyrosine hydroxylase, the rate limiting enzyme in catecholamine biosynthesis (see Axelrod 1971), its influence on resynthesis of catecholamine in the mammalian system is not clearly elucidated. Thus, Callingham and Mann (1962) found no difference in the replacement of adrenaline and noradrenaline between innervated and denervated adrenals of the rat after reserpine-induced depletion. Kroneberg and Schumann (1959), however, observed that in rabbits the rate of recovery of adrenaline was slower in the denervated adrenals than that in the intact ones. In contrast, Eränkö and Hopsu (1962) demonstrated that in rats the restitution of catecholamine after reserpine-induced depletion was
more rapid in denervated adrenals than in intact adrenals.

The role of splanchnic nerve on the restitution of adrenomedullary catecholamines after reserpine induced depletion is not apparently investigated in birds. Hence in this study, pigeon is selected as experimental model to explore the influence of splanchnic nerve on resynthesis of catecholamines in the avian group.

Materials and Methods

Fifty adult healthy pigeons (250–300 g) of both the sexes were bought from a local bird dealer and were acclimated to laboratory conditions for five days. Splanchnic denervation of the left adrenal gland was done in all the pigeons according to the method described earlier (Mahata & Ghosh 1985). They were kept for 7 days to facilitate the healing of the wounds. Right adrenal served as the innervated gland. Reserpine (0.8mg/100g body wt) was injected intraperitoneally to all the birds except the control group. The birds were killed by cervical dislocation after 1, 3, 6 and 9 days of reserpine administration. Control birds were also killed during the same period.

15μm frozen sections of right innervated and left denervated adrenals were used for histochemical analysis adopting the 'Modified Glyoxylic Acid Technique 'The SPG Method' of de la Torre and Surgeon (1976). The adrenals were quickly dissected out, weighed, and processed according to the method of Cox and Perhach (1973) and of Laverty and Taylor (1968) to determine the NE and E content spectrofluorometrically. The statistical analyses are done by Students t test.

Results

An intense green catecholamine (CAM) fluorescence was observed in the medullary cords of the right adrenal glands of the control pigeon (figure 1), irrespective of the sexes. No difference in CAM fluorescence was, however, encountered when compared to denervated (figure 2) and innervated (figure 1) adrenal glands.

The CAM fluorescence was found to have diminished markedly in both the innervated (figure 3) and denervated (figure 4) adrenal glands after one day post-reserpine injection. Denervated fluorescence seems to be more when compared with sections passing through comparable region in the innervated gland.

An increase in CAM fluorescence was observed in the innervated gland (figure 5) and to a lesser extent in the denervated adrenal glands (figure 6) 3 days after reserpine injection. CAM fluorescence was found to have increased further in the innervated gland after 6 days of reserpine injection. The denervated glands, however, did not show any appreciable increase in CAM fluorescence as compared to its innervated counterpart. The innervated adrenals 9 days after reserpine injection showed intense green CAM fluorescence (figure 7) comparable to that given in the control gland. However, in the denervated glands the increase in CAM fluorescence (figure 8) was not so pronounced as compared to its innervated counterpart after 9 days of reserpine injection.

In the innervated glands, the restitution of NE (figure 9) was completed 6 days after reserpine-induced depletion while resynthesis of E (figure 9) was not completed even 9 days after treatment. Resynthesis of total CAM (figure 9) was completed 9 days after reserpine-induced depletion when NE exceeds to that of the control value. In the denervated adrenals, the restitution of NE and E, however, was not completed even 9 days after drug-induced depletion.

Discussion

The present study reveals the accelerated
Figures 1–8 Catecholamine fluorescence micrographs of the innervated adrenal gland: control (figure 1), after reserpine treatment: for 1 day (figure 3, showing tremendous diminution of fluorescence); 3 days (figure 5, showing increased fluorescence); and 9 days (figure 7, showing pronounced fluorescence); and denervated adrenal gland: control (figure 2), after reserpine treatment: for 1 day (figure 4, showing pronounced diminution of fluorescence); 3 days (figure 6, showing increase in fluorescence); and 9 days (figure 8, showing increase in fluorescence but of a lesser extent than that of the innervated one). (Magnification of all photomicrographs (× 400 C, Cortex; M, Medulla)
Figure 9 Influence of splanchnic nerve on the resynthesis of catecholamine content following reserpine-induced depletion in the pigeon. Top figure represents resynthesis of NE in the innervated and denervated adrenal gland, showing 145% resynthesis of NE in the innervated gland in comparison to 48% in the denervated gland 9 days after depletion. Middle figure represents restitution of E in the innervated and denervated adrenal gland, showing 57% resynthesis of E in the innervated gland in comparison to 31% in the denervated gland 9 days after depletion. Bottom figure represents resynthesis of total CAM in the innervated and denervated gland, showing 98% restitution in the innervated gland and in contrast, only 39% resynthesis in the denervated gland 9 days after depletion. (A, B; C, D; and E, F refer to comparison in between the innervated and denervated gland 9 days after depletion).
resynthesis of NE in the innervated gland as compared to its denervated counterpart following reserpine-induced depletion in the pigeon. This increased rate of resynthesis of NE in the innervated gland may be due to the neural induction of tyrosine hydroxylase (cf. Axelrod 1971). It appears further that resynthesis of NE exceeds that of the control value 9 days after reserpine-induced depletion. In order to justify this finding, we may recall the work of Callingham and Mann (1958, 1962) who demonstrated that in the rat during hormonal resynthesis following reserpine-induced depletion NE concentration increases 300% above the original value by 7 days, and eventually decreases to reach its normal concentration.

The present investigation also reveals that restitution of both NE and E is much slower in the denervated adrenals as compared to the innervated counterpart. Therefore, there is no doubt that restitution process of catecholamine is under the control of cholinergic nerve fibres. The question then arises that which factor(s) influences resynthesis of catecholamines in the denervated gland. It is presumed that Adrenocorticocotrophin (ACTH) or any other hormone may be responsible for induction of the restitution of catecholamine in the denervated gland (cf. Carmichael 1983). With further analysis it appears that the rate of resynthesis of NE (approx. 48%) in the denervated adrenal gland is higher in comparison to restitution of E (approx. 31%). This higher rate of resynthesis of NE in the denervated gland is possibly due to the selective association of post-ganglionic intramedullary adrenergic nerve fibres with NE cells (cf. Prentice & Wood 1974, 1975). Obviously further studies are necessary to clarify these problems.

Acknowledgements
This work was supported by a grant from the University Grants Commission (F. 14-7/77, September 21, 1977).

References
Axelrod J 1971 Noradrenaline: fate and control of its biosynthesis; Science 173 598–606
Bygdeman S, Euler U S von and Håkfelt T 1976 Resynthesis of adrenaline in the rabbit’s adrenal medulla during insulin induced hypoglycemia; Acta Physiol. Scand. 49 21–28
Callingham B A and Mann M 1958 Adrenaline and noradrenaline content of the adrenal gland of the rat following depletion with reserpine; Nature 181 423–424
— and — 1962 Depletion and replacement of the adrenaline and noradrenaline contents of the rat adrenal gland following treatment with reserpine; Brit. J. Pharmacol. 18 138–149
Cox R H Jr and Perhach J L Jr 1973 A sensitive, rapid and simple method for the simultaneous spectrophotofluorometric determinations of norepinephrine, dopamine, 5-hydroxy-tryptamine and 5-hydroxy indole-acetic acid in discrete areas of brain; J. Neurochem. 20 1777–1780
Ernöö O and Hopsu V 1962 Effect of denervation on reserpine induced depletion and subsequent restitution of adrenaline and noradrenaline in the adrenal medulla of the rat; Ann. Med. Exp. Fenn. 40 134–145
Kroneberg G and Schumann H J 1959 Über die Bedeutung der Innervation für die Adrenalin-synthese im Nebennierenmark; Experientia (Basel) 15 234–235
Laverty R and Taylor K M 1968 The fluorometric assay of catecholamines and related compounds: improvements and extensions to the hydroxyindole technique; Anal. Biochem. 23 269–279
Mahata S K and Ghosh A 1985 Effect of denervation and/or reserpine induced changes on adrenomedullary catecholamines in pigeon: a fluorescence histochemical study; Basic Appl. Histochem. 29 331–336
Prentice F D and Wood J G 1974 Cytochemical localization of 5-Hydroxydopamine in adrenergic elements of cat adrenal medulla; Experientia 30 645–648
— and — 1975 Adrenergic innervation of cat adrenal medulla; Anat. Rec. 181 689–704
Torre J C de la and Surgeon J W 1976 A methodological approach to rapid and sensitive monoamine histofluorescence using a modified glyoxylic acid technique; The SPG method; Histochemistry 49 81–93