A DECREASE IN ACETYLCHOLINE CONTENT OF THE RAT VAS DEFERENS AFTER SURGICAL DENERVATION

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Acetylcholine contents of the rat vasa deferentia were measured after surgical denervation, daily reserpine treatment or 6-hydroxydopamine pretreatment, all of which are known to cause a decrease in norepinephrine contents. Denervation and reserpine treatment are known to cause nonspecific supersensitivity to agonists. Denervation caused a decrease in acetylcholine content but reserpine and 6-hydroxydopamine failed. It is suggested that surgical denervation causes adrenergic and cholinergic denervation and that acetylcholine content *per se* is not causally related to the appearance of nonspecific supersensitivity.

Keywords——acetylcholine; denervation; 6-hydroxydopamine; rat vas deferens; reserpine

Surgical denervation of the rat vas deferens produces a decrease in norepinephrine(NE) content and the nonspecific supersensitivity.^{1,2)} The denervation procedure of the rat vas deferens we have adopted is to desheath a part of this organ close to the seminal vesicle.¹⁾ Since acetylcholinesterase-containing neuron has its cell body close to the urethra,³⁾ it appeared to be necessary to examine whether or not the cholinergic nerve of the rat vas deferens remains intact after denervation procedure. Therefore, we decided to measure the acetylcholine(ACh) content of the vas deferens after the procedures which have been established to cause a decrease in NE content of this organ.

METHODS

Male Wistar rats weighing 250 to 320g at the start of the experiment were used. Rats were killed 7 days after surgical denervation,¹⁾ after treatment of reserpine(RSP) at 1 mg/kg/day for 6 days⁴⁾ or 7 days after the second injection of 6-hydroxydopamine hydrobromide(6-OHDA) (100 mg/kg *i.v.* and the next day, 250 mg/kg *i.v.*).⁵⁾ The vasa were dissected out. NE content was measured

fluorimetrically^{6,7)} and ACh content was measured by gas chromatography.⁸⁾

RESULTS

Surgical denervation, daily RSP treatment and 6-OHDA pretreatment decreased endogenous NE content of the rat vas deferens to 3.8%, 7.2% and 27.3%, respectively (Table I).

Of these procedures surgical denervation produced a decrease in ACh content of the vas deferens to 28.6% but RSP and 6-OHDA failed to produce any change in ACh content (Table I). After these procedures, wet weights of the vas deferens were not changed.

DISCUSSION

Nonspecific supersensitivity of the rat vas deferens, *i.e.*, leftward shift of the dose-response curve to various agonists such as NE and ACh, is known to be caused by surgical denervation¹⁾ or daily RSP treatment.⁴⁾ And it is also known that chemical sympathectomy by 6-OHDA causes supersensitivity to NE of postsynaptic origin.⁵⁾ Denervation causes a decrease in NE content and

TABLE I. Effect of Denervation, Reserpine(RSP) and 6-Hydroxydopamine(6-OHDA) on Norepinephrine(NE) and Acetylcholine(ACh) Content of the Rat Vas Deferens

	N ^{a)}	NE content nmol/g $\pm$ S.E.	N ^{a)}	ACh content nmol/g $\pm$ S.E.
Control	6	61.1 $\pm$ 3.0	5	9.8 $\pm$ 1.5
Denervated	6	2.3 $\pm$ 1.0 ^{b)}	5	2.8 $\pm$ 0.5 ^{c)}
Control	5	63.4 $\pm$ 4.6	8	9.0 $\pm$ 1.2
RSP	5	4.5 $\pm$ 1.2 ^{b)}	6	10.3 $\pm$ 1.4
Control	6	47.1 $\pm$ 6.6	7	7.2 $\pm$ 1.1
6-OHDA	5	12.9 $\pm$ 1.3 ^{b)}	6	6.2 $\pm$ 1.5

a) Number of observations: One vas deferens was used per one observation in the case of NE determination and two or three pooled vasa were used in ACh determination.

b) $p < 0.01$.

c) $p < 0.05$.

acetylcholinesterase activity.⁹⁾ But as acetylcholinesterase is not always a marker enzyme of cholinergic nerve¹⁰⁾ we have measured the tissue content of ACh. In the present study ACh content of the vas deferens was found to decrease after denervation, which suggests that cholinergic as well as adrenergic nerves were denervated by this procedure. This result coincides with the histochemical observation which shows the existence of ganglion cells of acetylcholinesterase-positive nerves close to the urethra.³⁾ Intravenous injection of 6-OHDA and daily injection of RSP are also known to cause a decrease in NE content of the vas deferens.^{4,5)} In this study, it is shown that ACh content is not affected by RSP or 6-OHDA. From these results, it is shown that a decrease in ACh content *per se* is not causally related to the appearance of the nonspecific supersensitivity of the rat vas deferens, although the participation of the cholinergic neuron is not clear at present.

REFERENCES

- 1) Y.Kasuya, K.Goto, H.Hashimoto, H.Watanabe, H.Munakata and M.Watanabe: Nonspecific denervation supersensitivity in the rat vas deferens 'in vitro', *European J. Pharmacol.*, **8**, 177—184 (1969).
- 2) A.T.Birmingham: Sympathetic denervation of the smooth muscle of the vas deferens, *J. Physiol.*, **206**, 645—661 (1970).
- 3) J.S.Dixon and J.A.Goshing: The distribution of autonomic nerves in the musculature of the rat vas deferens. A light and electron microscope investigation, *J. Comp. Neurol.* **146**, 175—188 (1972).
- 4) T.J-F. Lee, D.P.Westfall and W.W.Fleming: The correlation between spontaneous contractions and post-junctional supersensitivity of the smooth muscle of the rat vas deferens, *J. Pharmacol. Exp. Ther.*, **192**, 136—148 (1975).
- 5) D.P.Westfall and J.S.Feden: The effect of pretreatment with 6-hydroxydopamine on the norepinephrine concentration and sensitivity of the rat vas deferens, *European J. Pharmacol.*, **33**, 413—417 (1975).
- 6) M.K.Shellenberger and J.H.Gordon: A rapid, simplified procedure for simultaneous assay of norepinephrine, dopamine, and 5-hydroxytryptamine from discrete brain areas, *Anal. Biochem.*, **39**, 356—372 (1971).
- 7) T.Karasawa, K.Furukawa, K.Yoshida and M.Shimizu: A double column procedure for the simultaneous estimation of norepinephrine, normetanephrine, dopamine, 3-methoxytryptamine and 5-hydroxytryptamine in brain tissue, *Japan. J. Pharmacol.*, **25**, 727—736 (1975).
- 8) W.B.Stavinoha and S.T.Weintraub: Estimation of choline and acetylcholine in tissue by pyrolysis gas chromatography, *Anal. Chem.*, **46**, 757—760 (1974).
- 9) D.P.Westfall, J.J.McPhillips and D.J.Foley: Inhibition of cholinesterase activity after postganglionic denervation of the rat vas deferens: Evidence for prejunctional supersensitivity to acetylcholine, *J. Pharmacol. Exp. Ther.*, **189**, 493—498 (1974).
- 10) D.R.Mottram, C.Ivens, J.D.Lever and R.Presley: A fine structural and enzyme histochemical study of muscle innervation in the normal and 6-hydroxydopamine(6-OHDA)-treated rat vas deferens including a numerical assessment of axonal changes within 48 hours of a single injection of the drug, *Neuropharmacology*, **12**, 583—595 (1973).