Electron Microscopic Evidence that Bretylium and Pargyline Delay Adrenergic Nerve Degeneration After Sympathectomy of the Pineal Gland

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Summary. Seventeen and twenty four hours after sympathetic denervation, noradrenaline stores of the rat pineal gland were depleted to 50% and 10% of controls, respectively. Electron microscopic studies showed the coexistence of normal and altered nerve endings 17 h after denervation, while 24 h after denervation, only degenerated nerve terminals were observed.

Treatment with pargyline (512 μmol/kg) or bretylium (24 μmol/kg) significantly delayed the loss of noradrenaline from denervated glands. In pargyline treated rats, 17 h after denervation, noradrenaline stores were 90% of control glands. After bretylium, values obtained 24 h after denervation, declined to 36% of innervated glands. Persistence of neurotransmitter coincided with the presence of normal nerve endings as observed electron microscopically.

It is concluded that both, pargyline and bretylium, prolonged the survival of nerve endings severed from the cell body.

Key words: Nerve degeneration - Noradrenaline - Pargyline - Bretylium - Pineal gland

Introduction

The MAO inhibitor pargyline and the adrenergic neuron blockers bretylium and β-TM-10 [2-(2,6-dimethylphenoxy)-propyl trimethyl-ammonium chloride monohydrate] are able to change the time course of certain events associated with adrenergic nerve degeneration after sympathetic denervation. Thus, after administration of either of these drugs, the loss of noradrenaline is retarded (Benmiloud and Von Euler 1963), degeneration activity is postponed (Lundberg 1969; ArbilIla et al. 1977) and both impairment of neuronal uptake as well as development of prejunctional supersensitivity are also delayed (Arbillla et al. 1977, 1980). Therefore, these findings would indicate that the whole process of adrenergic nerve degeneration can be delayed by these drugs. However, in the cat's nictitating membrane electron micrographs revealed no difference in the rate of degeneration of nerve endings between animals that received β-TM-10 and controls (Pluchino et al. 1970).

The aim of the present study was to investigate whether the delay in the loss of noradrenaline after sympathetic denervation induced by either bretylium or pargyline is associated with ultrastructural signs of a delay of nerve degeneration. For this purpose, the pineal gland of the rat was selected as the target organ, since it possesses an extensive and well studied adrenergic innervation (Pellegrino de Iraldi and Zieher 1966).

Material and Methods

Female Wistar rats, weighing 180–230 g were used. Postganglionic sympathetic denervation of the pineal gland was achieved by bilateral removal of the superior cervical ganglia, under ether anaesthesia. Body temperature was prevented to fall during anaesthesia and recovery period (Stefano and Perec 1979). The following experimental groups were designed: 1) intact control animals; 2) rats sacrificed 17 h after denervation; 3) rats treated with pargyline and sacrificed 17 h after denervation; 4) rats killed 24 h after denervation; 5) rats treated with bretylium and killed 24 h after denervation.

Pargyline hydrochloride (512 μmol/kg) was injected intraperitoneally immediately after denervation; bretylium tosylate (24 μmol/kg) was administered subcutaneously 10 h after denervation (ArbilIla et al. 1980). All animals were killed by cervical dislocation; the pineal gland was removed and 5 to 7 glands were pooled for noradrenaline determination. Tissues were homogenized in 0.4 N HClO₄, containing 1 mg/ml Na₂EDTA and 1.25 mg/ml Na₂SO₄. The extracts were chromatographed on alumina columns (ArbilIla et al. 1977) and measured fluorimetrically (Laverty and Taylor 1968).

For electron microscopic studies 4 pineal glands from group 1, 6 from group 2, 4 from group 3, 5 from group 4 and 6 from group 5, were cut by half in order to obtain two blocks per gland. The material was fixed for 3 h in a phosphate buffered p-formaldehyde solution (Karnovsky 1965), post-fixed in 1% phosphate buffered osmium tetroxide dehydrated in ethanol and embedded in araldite. Each block was then trimmed keeping the central part of each half-gland to be studied. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with a Siemens Elmiskop I electron microscope. Counting of the sympathetic nerve endings was performed at a constant magnification of ×5,000 at the level of the screen. In order to analyze the characteristics of each nerve terminal, the magnifying glass of the electron microscope was used (×50,000). No less than 30 fields per gland were observed and micrographs were taken at

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different magnifications. A total of 836 nerve endings was recorded. The number of axons with normal appearance, partially damaged (nerve showing few vesicles and some damage in their axolemma and mitochondria), and totally damaged (lack of vesicles and presence of dense bodies) were added and their percent distribution calculated.

Results

Figure 1 shows that the endogenous noradrenaline content in control animals was reduced from 1.05 ± 0.12 ng/gland (6 to 7 glands were pooled per group, n = 5) to 0.54 ± 0.02 ng/gland (6 to 7 glands were pooled per group, n = 3) 17 h and to 0.10 ± 0.08 ng/gland (6–7 glands were pooled per group, n = 4) 24 h after denervation.

Seventeen hours after denervation the loss of noradrenaline was almost completely prevented by pargyline (0.95 ± 0.06 ng/gland, n = 4). Bretylium also delayed the loss of noradrenaline: 24 h after denervation the noradrenaline content was reduced to 36% of normal (0.38 ± 0.04 ng/gland, n = 4) in bretylium-treated animals, although noradrenaline levels were then very low (see above) in untreated animals.

The electron microscopic studies revealed numerous normal sympathetic nerve endings in the pineal gland of intact rats (Fig. 2a). Seventeen hours after denervation most of the nerve terminals showed degenerative changes mainly consisting of swelling, lack of vesicles, axolemma damage and presence of membranes and heterogenous dense bodies of lysosomal type. Table 1 shows that 67.6% of the nerve endings were totally degenerated (17.3 ± 1.8 nerve endings counted in 30 fields per gland) and 25.4% were partially degenerated (6.5 ± 1.6 nerve endings) while only 7% of normal nerve fibers (1.8 ± 0.6 nerve endings) coexisted with the degenerated ones (Fig. 2b).

In 24 h denervated glands a minimal percentage of normal nerve endings (3.8%) and numerous axons undergoing severe degenerative changes similar to those mentioned above were observed (Fig. 2d and Table 1). Denervated pineal glands from rats treated with either pargyline or bretylium showed a decrease of about 30% of normal nerve endings (controls: 40.7 ± 3.4; pargyline: 27.8 ± 1.3; bretylium: 29.7 ± 1.5 nerve endings counted in 30 fields per gland, Table 1, Fig. 2c,e,f). However, some sympathetic nerve fibers with varying degrees of degeneration were also seen in glands treated with either pargyline or bretylium (Table 1).

Discussion

The present results show that in the pineal gland noradrenaline depletion after postganglionic sympathetic denervation follows a time course similar to that described in the submaxillary gland (Stefano et al. 1974). Moreover, the delay in noradrenaline loss induced by either pargyline or bretylium treatment was also similar to that reported previously for the submaxillary gland (Arbilla et al. 1977, 1980). Seventeen hours after denervation, when the gland's noradrenaline stores were depleted by about 50%, the electron micrographs showed the coexistence of normal and degenerated nerve endings. Twenty four hours after denervation, when the gland's noradrenaline levels were found to be less than 10% of control values, mainly degenerated nerve terminals were observed.

After administration of either pargyline or bretylium, not only the endogenous noradrenaline values of denervated glands were higher than those of untreated denervated rats, but also a higher proportion of nerve terminals with normal appearance was present in the drug-treated than in untreated animals. These findings confirm our previous suggestion that the persistence of neurotransmitter stores subsequent to denervation is an indication of the presence of normal nerve terminals (Arbilla et al. 1980). Therefore, it can be assumed that the effects induced by these drugs are related to an actual delay in the process of structural nerve degeneration and not solely to the prevention of noradrenaline from deamination in degenerating nerve terminals.