EFFECT OF TRIFLUOPERAZINE ON ULTRASTRUCTURE OF GIGANTOCELLULAR NEURONS OF THE RETICULAR FORMATION OF THE RAT BRAIN

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The ultrastructure of neurons of the reticular formation of the rat medulla was investigated at various times after administration of trifluoperazine in doses of 1, 5, and 10 mg/kg. The earliest changes were found in the mitochondria. Later these were joined by marked hyperplasia of membranes of the smooth endoplasmic reticulum and Golgi apparatus, accompanied by microvesiculation.

Despite the wide use of neuroleptics in the treatment of many mental diseases, their effect on the ultrastructure of the brain neurons has received little attention. One of the most active neuroleptics of the phenothiazine series, trifluoperazine, has been studied from this standpoint only by optical microscopy [3, 10, 12].

In the present investigation the ultrastructure of neurons in the gigantocellular region of the reticular formation was investigated during the action of trifluoperazine. The bulbar reticular formation was chosen for study because it is an important point of application of neuroleptics [1, 13].

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 180-200 g. Trifluoperazine was injected intraperitoneally in doses of 1, 5, and 10 mg/kg body weight. The brain was fixed after 3, 8, and 24 h by perfusion with glutaraldehyde followed by treatment with osmium tetroxide, and was then embedded in araldite. Sections were cut on the LKB ultratome, shadow-cast with uranyl acetate and lead citrate, and studied in the UÉMV-100V electron microscope. The term gigantocellular region of the reticular formation is taken to mean the nuclei reticularis gigantocellularis et reticularis pontis caudalis [2].

EXPERIMENTAL RESULTS

Analysis of the electron micrographs showed that 3, 8, and 24 h after administration of trifluoperazine certain reactive changes were visible in the membranous structures of the neurons: the mitochondria, Golgi apparatus, granular and agranular endoplasmic reticulum, and other formations. During the first few hours after injection of the neuroleptic in a dose of 1 mg/kg these changes were less marked and, as a rule, affected only individual structures of single cells, but with an increase in the duration of action and in the dose of the drug, they appeared more clearly. Changes arising after injection of trifluoperazine in doses of 5 and 10 mg/kg differed only very slightly. The nucleus of the neuron showed no visible changes during the first few hours after injection of the neuroleptic. A very slight increase in translucency of the nuclear contents, and accumulation of chromatin in the form of closely packed large and small granules were observed only in a few neurons in the later periods. The nuclear membrane formed invaginations, frequently at

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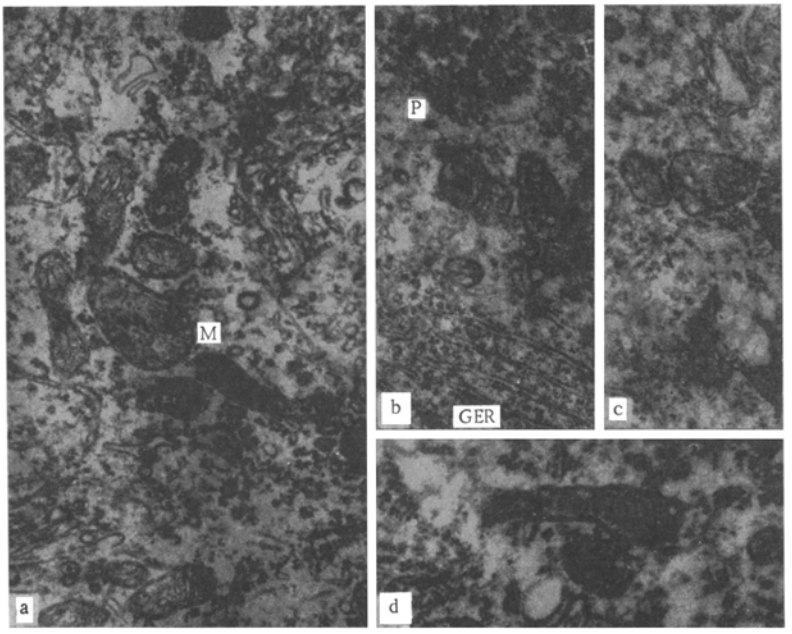

Fig. 1. Submicroscopic changes in neurons of reticular formation after injection of trifluoperazine: a) Mitochondria after injection of trifluoperazine. Organelles are visible but their cristae can no longer be seen. In some mitochondria the intercristal spaces are widened. Mitochondrion with enlarged cross section visible in center, 40,000×. b) Mitochondrion with septum in center, 38,000×. c, d) Different shapes of buds from mitochondria: c) 38,000×; d) 45,000×.

several places at once. Ribosomes and, less frequently, mitochondria, lysosomes, or vacuoles, were visible in the invaginated cytoplasm.

In some cases the space between the layers of the nuclear membrane was somewhat widened, the outline of the inner layer was wavy in character, and the number of pores in the nuclear membrane was increased.

The earliest reactive changes were found in the mitochondria. For example, 3 h after injection of the neuroleptic in a dose of 5 mg/kg, besides mitochondria clearly outlined with a double membrane, with well-defined cristae, and with a finely granular matrix, other organelles with swollen inner and outer membranes, with irregularly arranged cristae or, in some cases, with no visible cristae, were observed more frequently than in the control. Their inner structure consisted of a central homogeneous osmiophilic mass bounded by a double membrane (Fig. 1a). In some mitochondria with intact cristae, the spaces between the cristae were widened. After 8 and 24 h, these changes showed virtually no progression, but the mitochondria had become more heterogeneous: the transverse diameter of the sections through the organelles in many cases was greater than normal, and some large mitochondria with numerous cristae had appeared. After 8 and 24 h, many sections through the organelles were oval or circular in shape (Fig. 2a), whereas in intact animals elongated mitochondria were more common.

Mitochondria with various forms of hour-glass constrictions could be seen, and sometimes one mitochondria was apparently dividing into two daughter structures containing a common double membrane, but also surrounded by their own membrane (Fig. 1B). In some cases daughter mitochondria can evidently be formed by budding from the side (Fig. 1c, d). These mitochondria are very similar in appearance to dividing organelles. "Degenerative forms" of mitochondria were also found, with the appearance of dense osmiophilic bodies in which indistinct cristae were sometimes present.

A particularly characteristic feature of neurons of the gigantocellular region was the considerable development of the membranes of the granular endoplasmic reticulum. The agranular endoplasmic reticulum in the intact neurons was less well developed and consisted of separate closed cavities in the membranes

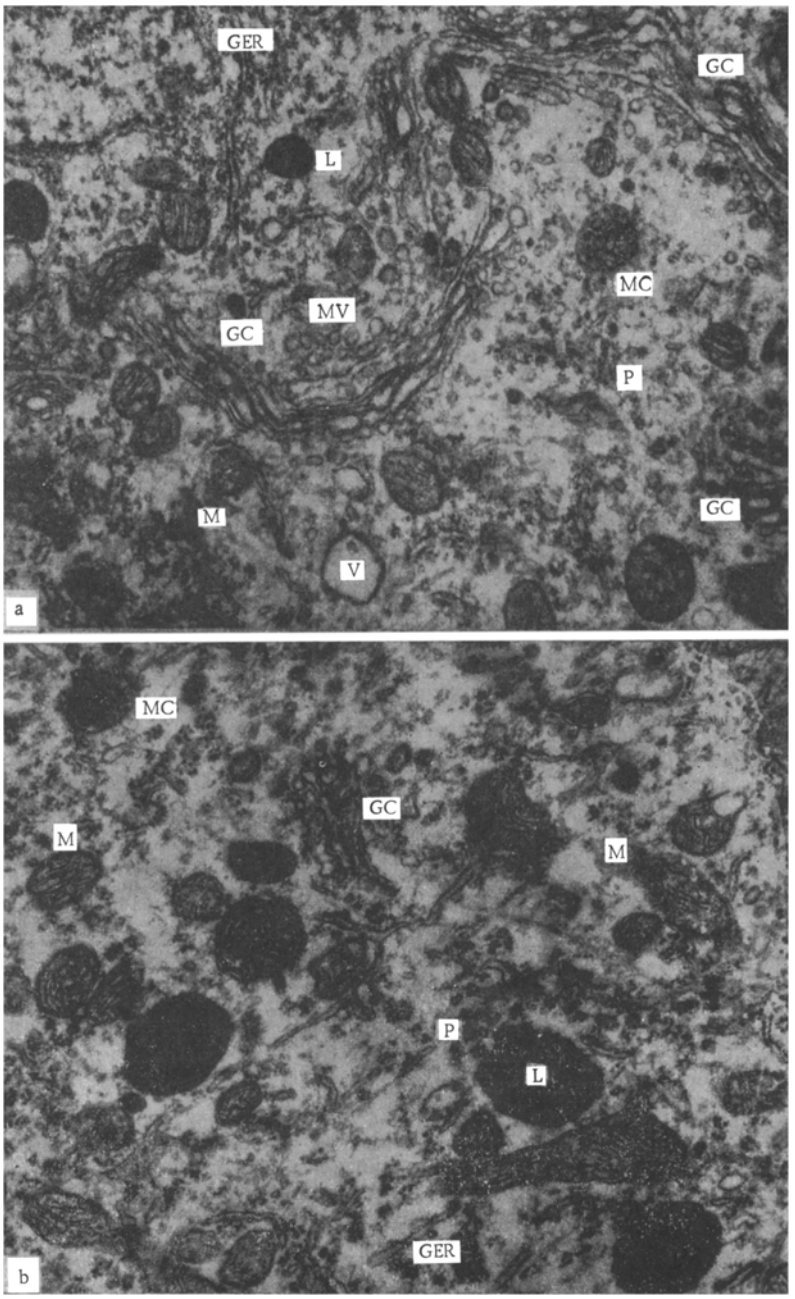

Fig. 2. Hyperplasia of Golgi apparatus (a) and increase in number of lysosomes (b) after injection of trifluoperazine: M) mitochondria, P) polysomes, GER) granular endoplasmic reticulum. In a: MC) microcorpuscle; V) vacuole; MV) microvesicles; GC) Golgi complex; remainder of legend as in Fig. 1. 40,000×. In b: L) lysosomes; remainder of legend the same. 45,000×.

with the appearance of cisterns, vesicles, and tubules. Changes in the granular endoplasmic reticulum by the action of trifluoperazine were ill defined, and appeared after 8 and 24 h as a slight narrowing of the zones of Nissl's substance and in some cases disorganization of the parallel rows of membranes and some decrease in the number of polysomes. By this time hyperplasia of the membranes of the smooth reticulum, accompanied by the appearance of flat cisterns, tubules, and larger and smaller vacuoles, was visible. These vacuoles were close to the elements of the Golgi apparatus or in contact with them. The Golgi apparatus also was hyperplastic, and in some fields of vision up to eight or 10 Golgi complexes could be seen in

the cytoplasm, compared with two or three in intact animals (Fig. 2a). Frequently the Golgi complexes were arranged in a horseshoe. Their component elements increased in length and were surrounded by numerous vesicles and cisterns. Sometimes the microvesiculation process was well defined and small vesicles occupied large areas of the sections. Frequently lysosomes were present near them, and their number was also increased (Fig. 2b). Individual lysosomes contained foci of translucency, located eccentrically. In some cases structures similar to lysosomes, but without a clear boundary membrane, and called "foci of degeneration" [4], were found. In the cytoplasm of the cells, microcorpuscles (Fig. 2a) and also structures resembling vesicles, about 1000 A in diameter, could be seen. Osmiophilic granules, similar to ribosomes, were attached to the outer surface of these structures. Such vesicles have been described in nerve cells [4, 15]. Frequently structures consisting of smooth membranes, and resembling transversely divided medullated fibers, appeared in the cytoplasm.

Hence, among the submicroscopic changes taking place after injection of trifluoperazine, the most prominent was the marked sensitivity of the mitochondrial apparatus of the cell, which responded rapidly by a change in the internal structure of some organelles and also by a change in the shape of the mitochondria (with the appearance of more circular and, sometimes, of hypertrophied forms, and with an increase in the diameter of cross section of the organelles). Similar changes in the internal structure of the mitochondria of neurons have been described by the action of chlorpromazine [8].

In many cases the mitochondria are reactive structures [6, 9], and a change in their structure may be accompanied by disturbances of respiratory, phosphorylating, and other functions [5]. It can accordingly be postulated that the action of trifluoperazine is accompanied by a change in some mitochondrial functions. This is in agreement with published reports of the effect of phenothiazine derivatives on the activity of respiratory enzymes [7, 11, 14]. The hyperplasia of the membranes of the smooth endoplasmic reticulum and Golgi apparatus observed in the later stages, accompanied by microvesiculation, an increase in the number of lysosomes, and the appearance of foci of degeneration may also indicate a considerable degree of modification of metabolic processes in the nerve cells under the influence of the neuroleptic.

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