EFFECT OF NEUROLEPTICS OF THE PHENOTHIAZINE SERIES ON FIRING RATE AND ULTRASTRUCTURE OF RABBIT CORTICAL NEURONS

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In experiments on rabbits chlorpromazine, in a dose of 5 mg/kg, caused deaggregation of the polysomes, dilatation of the cisterns of the endoplasmic reticulum, hypertrophy of the Golgi apparatus, and swelling of the mitochondria in most cortical neurons. These ultrastructural changes were accompanied by inhibition of unit activity. Trifluoperazine, in the same dose, unlike chlorpromazine led to condensation of the mitochondrial matrix of some neurons, with a corresponding increase in the firing rate of 28% of cortical cells. This correlation between changes in unit activity and neuronal ultrastructure is examined in connection with differences in the spectrum of psychotropic activity of the two phenothiazine derivatives with neuroleptic properties.

KEY WORDS: action of chlorpromazine; cerebral cortex.

Neuroleptics of the phenothiazine series differ in the spectrum of their clinical action. In experiments on rats the writers showed previously that trifluoperazine which, unlike chlorpromazine, has a wide active dose range (1-10 mg/kg), if given as a single dose or repeatedly over a period of time induces heterogeneous changes in the mitochondria of cortical neurons [3, 4]. A similar response of the mitochondria was found in the gigantocellular nucleus of the brain-stem reticular formation in rats [2].

To interpret these differences in the ultrastructural changes arising under the influence of chlorpromazine and trifluoperazine the action of these neuroleptics was studied on the structure and function of cortical neurons in rabbits.

EXPERIMENTAL METHOD

Chlorpromazine and trifluoperazine were injected intraperitoneally into male rabbits weighing 2.8-3.4 kg in a dose of 5 mg/kg (2.5% solution). Control animals received physiological saline. For electron-microscopic study 26 rabbits were decapitated 4 h after the injection. Pieces of the visual cortex were fixed with osmium tetroxide, dehydrated, and embedded in Araldite. Sections were cut on the LKB ultratome and studied in the JEM-6Y electron microscope. Electron micrographs of neurons from the control and experimental animals were classified according to features listed in Fig. 1.

For electrophysiological investigation 49 rabbits were scalped under local anesthesia 24 h before the experiment and the base of a microelectrode holder secured to the cranial bones. On the day of the experiment the skull was trephined and the burr-hole 3 mm in diameter was filled with agar-agar. The drugs or physiological saline were injected 3 h before the experiment began.

Visual cortical unit activity was recorded by tungsten extracellular microelectrodes and assessed from the mean firing rate. Only those neurons whose activity was recorded not later than 5 h after injection of the drugs or saline were analyzed.

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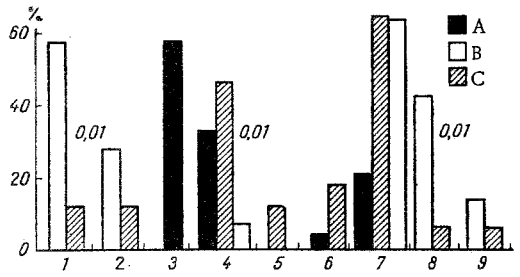

Fig. 1. State of some organelles of neurons after administration of chlorpromazine (B) and trifluoperazine (C). A) Control. Ordinate, percentage of neurons containing mitochondria (1-6) with swollen matrix and with reduced (1) or normal number of cristae (2), without condensation or swelling of the matrix and with a normal number of cristae (3), with condensation of the matrix and intact cristae (4), with ill-defined cristae and condensed matrix (5), with widened cristae and a sharp increase in density of the matrix (6), containing a dilated endoplasmic reticulum (7), hypertrophied Golgi apparatus (8), or an increased number of lysosomes (9). Numbers on right of columns denote level of significance of differences between action of chlorpromazine and trifluoperazine.

TABLE 1. Changes in Structure of Polysomes of Cortical Neurons

Treatment	No. of ribosomes in polysome and relative percentage of polysomes				Mean number of ribosomes in polysome and error of means
	1–2	3–4	5–6	7–8	
Control	18,6	41,0	36,8	2,8	3,9±0,03
Trifluoper.	33,4	46,8	17,8	1,0	3,1±0,06
Chlorprom.	45,0	42,4	13,2	0	2,9±0,11

EXPERIMENTAL RESULTS AND DISCUSSION

The ultrastructure of the overwhelming majority of neurons in the control animals corresponded to that described previously [7].

Both neuroleptics evoked similar changes in the neurons, consisting of dilatation of the cisterns of the endoplasmic reticulum, hypertrophy of the Golgi apparatus, etc. (Fig. 1). Chlorpromazine led more frequently to hypertrophy of the Golgi apparatus and to an increase in the number of lysosomes, possibly as the result of its stronger toxic effect.

Changes in the polysomes in the cytoplasm of the neurons were qualitatively identical. Both drugs caused a relative increase in the number of free ribosomes and polysome-dimers ($P < 0.01$) and also a decrease in the number of polysomes consisting of five or six ribosomes (Table 1). The intensity of protein synthesis is proportional to the number of ribosomes per polysome [6]. Earlier biochemical investigations demonstrated the inhibition of protein synthesis by chlorpromazine [13]. Deaggregation of the polysomes by the action of neuroleptics could also be a structural mechanism of the reduction in protein synthesis in the CNS. The intensity of this process was increased by chlorpromazine.

Differences in the spectrum of therapeutic activity of the two neuroleptics focus attention on the response of the mitochondria. After administration of chlorpromazine the mitochondria of nearly all neurons were comparatively round in shape, swollen, and with a clear matrix. Swelling of the mitochondria in more than half of the cells was accompanied by a reduction in the number of cristae (Figs. 1 and 2a). It is interesting to note that swelling of the mitochondria in some neurons was accompanied by a decrease in their number. Only very few neurons had mitochondria with a condensed matrix and intact cristae.

Under the influence of trifluoperazine the mitochondria of a comparatively small group of cells also were swollen and had a normal or reduced number of cristae (Fig. 1). Most neurons contained irregularly shaped mitochondria whose matrix was condensed to a varied degree. Most of these neurons had mitochondria with clearly outlined cristae (Figs. 1 and 2b). Neurons with such mitochondria also were seen in the control group. In other cells the mitochondrial cristae were ill-defined against the background of the dense matrix (Figs. 1 and 2c). Finally, some organelles had an extremely dense matrix and pale cristae (Figs. 1 and 2d).

Correlation between the structure and function of mitochondria has frequently been demonstrated. The state of their matrix and cristae is particularly important for functional interpretation [8, 9]. Swelling of mitochondria produced by widely different agents [5, 10, 12] is accompanied by a decrease in their phosphorylating activity. These observations have been made chiefly in vitro. With regard to the cellular mechanisms all that can be suggested is that the almost total swelling of the mitochondria under the influence of chlorpromazine may reduce the energy supply of the neurons and thus inhibit their function.

The structural heterogeneity of the mitochondria in response to the action of trifluoperazine suggests their functional heterogeneity also, for it has been shown both in vitro [9] and in situ [11] that trans-

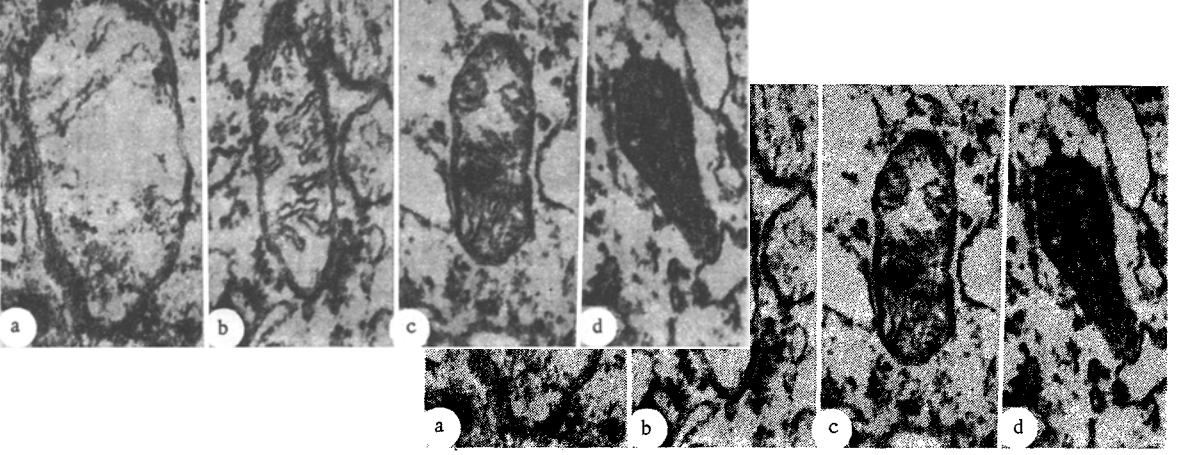

Fig. 2. Mitochondria after administration of chlorpromazine and trifluoperazine: a) mitochondrion with clear matrix and reduced number of cristae (chlorpromazine; 15,000×); b) mitochondrion with reduced density of matrix and intact cristae (trifluoperazine; 15,000×); c) mitochondrion with condensed matrix and indistinctly outlined cristae (trifluoperazine; 15,000×); d) mitochondrion with dense matrix and pale cristae (trifluoperazine; 12,000×).

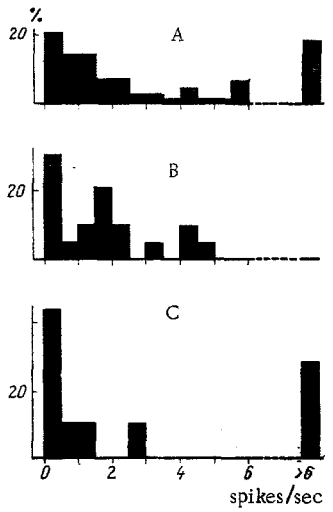

Fig. 3. Distribution of neurons by mean firing rate: A) control; B) chlorpromazine; C) trifluoperazine. Abscissa, firing rate (spikes/sec); ordinate, percentage of neurons.

formation of mitochondria from an "orthodox" (swollen) to a "condensed" state of the matrix is associated with induction of oxidative phosphorylation. If mitochondria with a condensed matrix after administration of trifluoperazine can be interpreted as organelles synthesizing ATP, there is reason to suggest that some neurons may be activated under the influence of this drug.

This hypothesis was tested by an electrophysiological investigation.

The first feature to be noted was that unit activity of the visual cortex of the intact rabbit brain varied from 0.1 to 17.3, with a mean value of 3.43 ± 0.41 spikes/sec. The distribution of the neurons by mean firing rate had a mode at below 1 spike/sec and thereafter it diminished almost exponentially.

After administration of chlorpromazine the mean firing rate fell to 1.77 ± 0.34 spikes/sec and no neuron discharged at a frequency greater than 5 spikes/sec (Fig. 3).

By contrast with the control cells and the neurons under the influence of chlorpromazine, which behaved like homogeneous populations, under the influence of trifluoperazine the cortical neurons formed two distinct groups on the basis of their mean firing rate ($P < 0.01$; Fig. 3). Cells (72%) with a firing rate of under 3 spikes/sec (mean 0.83) formed one group. The other group (28%) of neurons had a firing rate of between 6 and 26 spikes/sec (mean 11.13).

Against this background of reduced spike discharge of most neurons under the influence of trifluoperazine, in some cells the firing rate, on the contrary, increased. These observations suggest that the increased discharge activity of some neurons could be connected with the presence of highly active mitochondria in them.

The absence of mitochondria of this type after administration of chlorpromazine could be the factor determining the reduced unit activity observed in the rabbit visual cortex. This could account, to some extent perhaps, for the "repressive" action of chlorpromazine in clinical practice. Unlike chlorpromazine, trifluoperazine not only has higher antipsychotic activity but can also activate or "energize" patients [1]. This clinical feature of trifluoperazine may perhaps be connected with its ability to activate a small proportion of neurons while depressing the great majority.

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