SELECTIVE EFFECT OF TRIFLUOPERAZINE ON VARIOUS PARTS OF THE RETICULAR FORMATION OF THE RAT BRAIN

V. A. Markin
and V. A. Arefolov

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Trifluoperazine (stelazine), in doses of 1-20 mg/kg, when given as a single intraperitoneal injection causes marked inhibition of activity of various respiratory enzymes and changes in neuronal ultrastructure in the region of the medullary reticular formation (RF) while leaving the mesencephalic RF relatively intact. The results demonstrate reduced functional activity of the caudal divisions of the brain-stem RF and possible activation of its mesencephalic part under the influence of the drug.

The role of the brain-stem reticular formation and, in particular, of its various regions in the mechanism of action of neuroleptics have not yet been explained.

In this investigation an attempt was made to demonstrate effects of trifluoperazine, a specific neuroleptic, on various levels of the reticular formation (RF).

EXPERIMENTAL METHOD

Experiments were carried out on 150 male albino rats weighing 180-220 g. The animals' brains were studied 1, 3, 8, and 24 h after a single intraperitoneal injection of trifluoperazine in doses of 1, 5, 10, and 20 mg/kg. The rats were killed by decapitation. In histochemical experiments the corresponding parts of the brain from experimental and control animals were mounted in the same block. Sections were cut to a thickness of 20 μ in a cryostat at −10°C. Activity of the following enzymes was studied: succinate dehydrogenase (SDH) by the method of Nachlas et al. [11], $NAD \cdot H_2$ and $NADP \cdot H_2$-dehydrogenases by the method of Scarpelli et al. [13], mitochondrial α-glycerophosphate dehydrogenase (α-GDPH) by the method of Nachlas et al. in Kul'tas' modification [1], glutamate (GDH), isocitrate (IDH), malate (MDH), and lactate (LDH) dehydrogenases by the method of Hess et al. [10] in Pearse's modification [12].

For electron microscopy the brain was fixed by perfusion with glutaraldehyde followed by postfixation with osmium tetroxide, and embedded in Araldite. Sections were cut in the LKB ultratome, shadowed with uranyl acetate and lead citrate, and studied in the UÉMV-100 B microscope.

EXPERIMENTAL RESULTS

Under the influence of trifluoperazine a marked decrease in the activity of flavin and NAD-dependent dehydrogenases took place in the medullary RF and was more clearly observed after injection of the drug in doses of 5, 10, and 20 mg/kg (Table 1). Within this range of doses the inhibitory effect of trifluoperazine was exhibited to virtually an equal degree. As Table 1 shows, flavin enzymes (SDH, α-GPDH, $NAD \cdot H_2$ and $NADP \cdot H_2$ dehydrogenases) were more sensitive to the effect of trifluoperazine than the NAD-

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TABLE 1. Changes in Activity of Flavin and NAD-dependent Hydrogenases in Medullary Reticular Formation of Rats After a Single Injection of Trifluoperazine

Enzymes	Trifluoperazine					
	1 mg/kg			5, 10, 20 mg/kg		
	1 h	3 h	24 h	1 h	3 h	24 h
SDH, α-GDPH, NAD · H_2 and NADP · H_2 dehydrogenases	+	++	++(+)	+++	++++	++++
GDH, IDH, MDH, LDH	(+)	+	+(+)	+	++	+++

Legend. ++++ very high degree of enzyme activity; +++ high degree; ++ moderate degree, + weak enzyme activity; (+) tendency toward decrease in enzyme activity

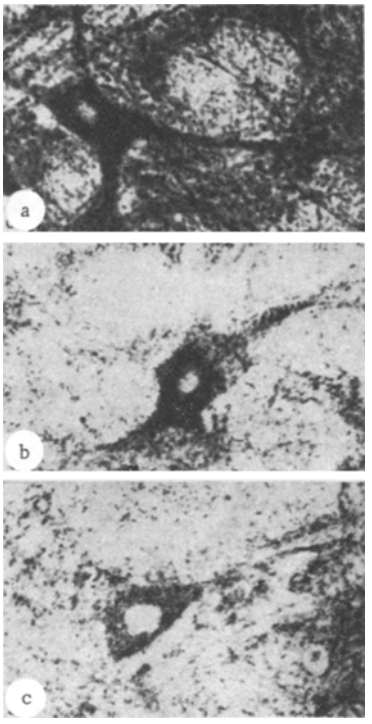

Fig. 1. Changes in NAD · H_2 dehydrogenase activity in medullary RF of rats after injection of trifluoperazine in a dose of 5 mg/kg: a) control; b) 3 h, c) 24 h after injection of drug, 420 ×.

dependent dehydrogenases, possibly due to the chemical affinity of the prosthetic group of the flavin enzymes for the phenothiazine ring of trifluoperazine [14]. The histochemical changes revealed in the medullary RF were chiefly located in small and medium-sized neurons, as well as in their processes (Fig. 1). Large and giant neurons were resistant to the drug. It is interesting to note that the deposition of diformazan in the dendrites and axons was reduced in the direction from the periphery toward the center, whereas in the bodies of the neurons the process took place in the opposite direction: from the nucleus toward the outer membrane. It should also be noted that dehydrogenase activity was inhibited predominantly in the medial zone of the medullary RF and, in particular, in the region of the raphe. The enzymic changes progressed with time, so that their intensity was higher 24 h after injection than 3 h after injection of trifluoperazine. Conversely, in the mesencephalic RF the dehydrogenase activity was virtually indistinguishable from the control after administration of trifluoperazine, except for an extremely slight decrease in activity of the flavin dehydrogenases after injection of the drug in a dose of 20 mg/kg.

Investigation of the ultrastructure of the medullary RF 3 h after injection of trifluoperazine showed changes in the mitochondria consisting of swelling of the mitochondrial membranes, disorganization of cristae and, in some cases, their total disappearance. Such mitochondria had a homogeneous osmiophilic matrix, but they were surrounded by a clearly defined double membrane. Side by side with altered mitochondria, some organelles were completely intact. After 8 and 24 h, besides the changes already described, some neurons showed narrowing of the zones of the granular reticulum and polysomes, disorganization of the series of parallel membranes, and an increase in the number of lysosomes (Fig. 2). In addition, hyperplasia of the membranes of the smooth reticulum and Golgi complex was observed and was accompanied by the formation of vesicles and vacuoles. Ultrastructural changes were more clearly visible after an increase in the dose of the drug, although no direct dependence of this effect could be detected. In the mesencephalic RF, on the other hand, the neuronal structures remained intact after injection of trifluoperazine. In fact, in the large cells there was actually some increase in the number of mitochondria together with widening of the zones of polysomes and granular endoplasmic reticulum. In the later stages, these changes were accompanied by moderately severe hyperplasia of the elements of the Golgi apparatus.

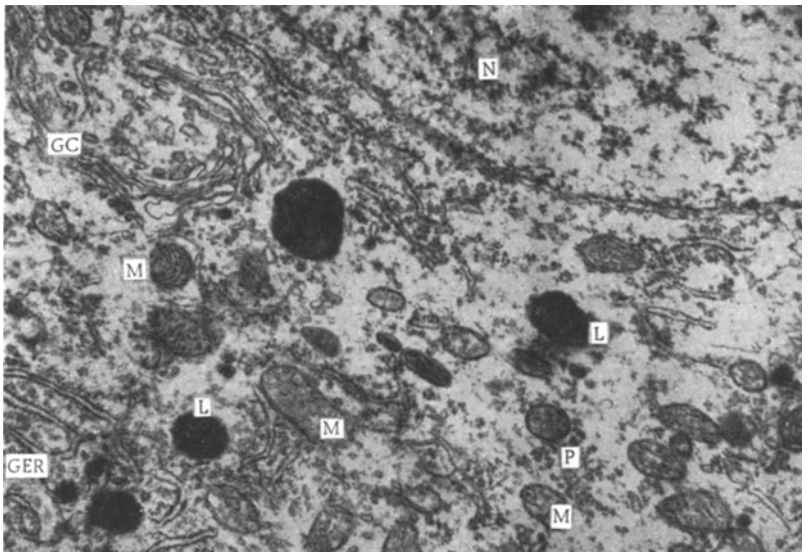

Fig. 2. Ultrastructure of medullary neurons of rats 8 h after injection of trifluoperazine in a dose of 5 mg/kg: N) nucleus; M) mitochondria; GER) granular endoplasmic reticulum; P) polysomes; GC) Golgi complex; L) lysosomes; 35,000 ×.

The histochemical and ultrastructural changes thus demonstrate the selective action of trifluoperazine on the medullary reticular formation compared with the mesencephalic RF. The decrease in activity of certain respiratory enzymes and the observed changes in the fine structure of the neurons, especially of the mitochondria, which contain the greater part of the dehydrogenases studied, reflect inhibition of energy, and, possibly, other types of metabolism. This suggests that the functional activity of caudal regions of the brain-stem RF is depressed by trifluoperazine. Meanwhile the function of the mesencephalic part of the RF remains evidently at its previous level, or is actually increased to a small extent, for the respiratory enzymes and cell ultrastructure in this part show no significant changes, and in some neurons the number of mitochondria is increased and the zones of the granular reticulum and polysomes are widened. This hypothesis is in agreement with electrophysiological [4, 6] and biochemical [5, 7] findings to the effect that stellazine increases the reflex activity of single neurons and stimulates oxidative phosphorylation and respiration in the region of the mesencephalic RF. The caudal part of the brain-stem RF thus has an inhibitory function relative to the sensomotor cortex [8, 9], and the depression or removal of the inhibitory effects by trifluoperazine is probably accompanied by activation or "liberation" of this part of the cortex which, in turn, may exert a descending inhibitory effect on the medullary RF. This could explain the fact that the histochemical and ultrastructural changes observed in the medullary RF are potentiated in time, whereas in other parts of the brain dehydrogenase activity is almost completely restored 24 h after injection of trifluoperazine [2, 3].

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