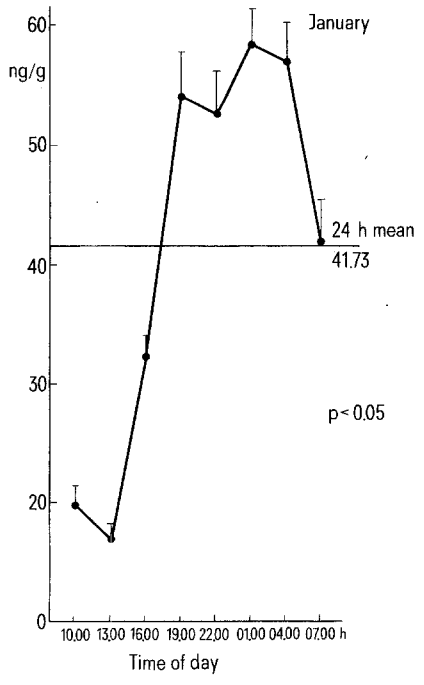

Circadian variation in β-glucuronidase activity in male rat liver. The activity is expressed in ng/g liver tissue. Each point represents the data from 6 animals with $\pm$ SD.

distribution patterns of all lysosomal enzymes are not identical[7]. This also holds good when the histochemical distribution patterns of these enzymes are compared[3]. Though both acid phosphatase and β-glucuronidase are accepted as the marker enzymes for the lysosomal study, it can be seen from the present study that their activity pattern during a 24-h period is quite different. This confirms once more the heterogenous nature of lysosomes. Therefore the study of lysosomal function through one of the lysosomal enzymes might not give a clear picture of their functional role. Moreover, as the main function of the lysosomal enzymes is to detoxicate toxic materials, the circadian variation in lysosomal enzyme activity is of considerable importance from this point of view. The temporal oscillation of the enzyme activities might influence the body's detoxicating mechanism.

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Modifications of female sex chromatin (Barr body) in rat neurons after reserpine administration[1]

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Summary. 3 groups of rats were sacrificed 30 min, 4 h and 24 h after reserpine (10 mg/kg, i.p.) injection. Toluidine blue stained sections showed that in the motor neurons of the spinal cord the drug, at 4 h, had induced a migration of the Barr body from the nucleolus to the nuclear membrane and an increase in its size and RNA concentration. From our findings we suggest that reserpine may have an activating role on X-linked genes.

Previous studies on the effect of psychoactive drugs at the cellular level have shown changes in nucleic acid histochemistry in rat sympathetic neurons after acute administration of reserpine, which involved the emergence of new RNA both in the nucleus and cytoplasm[5]. Furthermore, changes in nucleoprotein ultrastructure have also been observed in peripheral blood cells of schizophrenic patients after the administration of neuroleptics[6]. Alterations in metaphase chromosome structure have also been induced in vitro by chlorpromazine[7]. The purpose of the present study was to investigate whether the increase in RNA found in sympathetic neurons after reserpine administration[5] was a result of an initial interaction of this drug with the chromatin. To avoid a possible indirect effect through biogenic amines, we chose to study the motor neurons of the spinal cord.

Materials and methods. 18 adult female albino rats, weighing about 200 g each, were divided into 6 groups. 3 groups of rats, given reserpine (10 mg/kg, i.p.), were sacrificed at 30 min, 4 h and 24 h after injection. The remaining 3 groups, given saline and sacrificed at the same time intervals, served as controls. Segments of spinal cord were fixed in 10% formalin. Paraffin sections were stained with Toluidine blue for RNA and with the Feulgen method for DNA.

Results. Observation of the animals indicated that sedation was already evident at 30 min and maximal at 4 h. The effect of the drug had almost been dissipated by 24 h. In Feulgen stained preparations, the sex chromatin (Barr body) of the motor neurons in the controls appeared as a small red granule adjacent to the nucleolus. At 30 min reserpine had induced a partial euchromatization of the Barr body; this decondensation persisted at 4 h. In addition, a migration of this body was observed from its typical position on the nucleolus to the nuclear membrane at 4 h, while at 24 h it was found again, in most neurons, next to the nucleolus. In the Toluidine blue stained sections the motor horn neurons in the control (figure 1) were characterized by clear nuclei, displaying a faintly stained sex chromatin next to the nucleolus, and distinct large Nissl bodies in the perikarya and the dendrites. At 30 min after reserpine administration (figure 2) diffuse RNA occupied the entire nuclear area and was also found in increased concentration in the cytoplasm and on the Nissl bodies. The Barr body, still faintly stained, was located at a distance from the nucleolus. At 4 h (figure 3), when maximal sedation of the animals was observed, the Barr body was intensely stained, noticeably large in size and adjacent to the nuclear membrane. Other striking changes

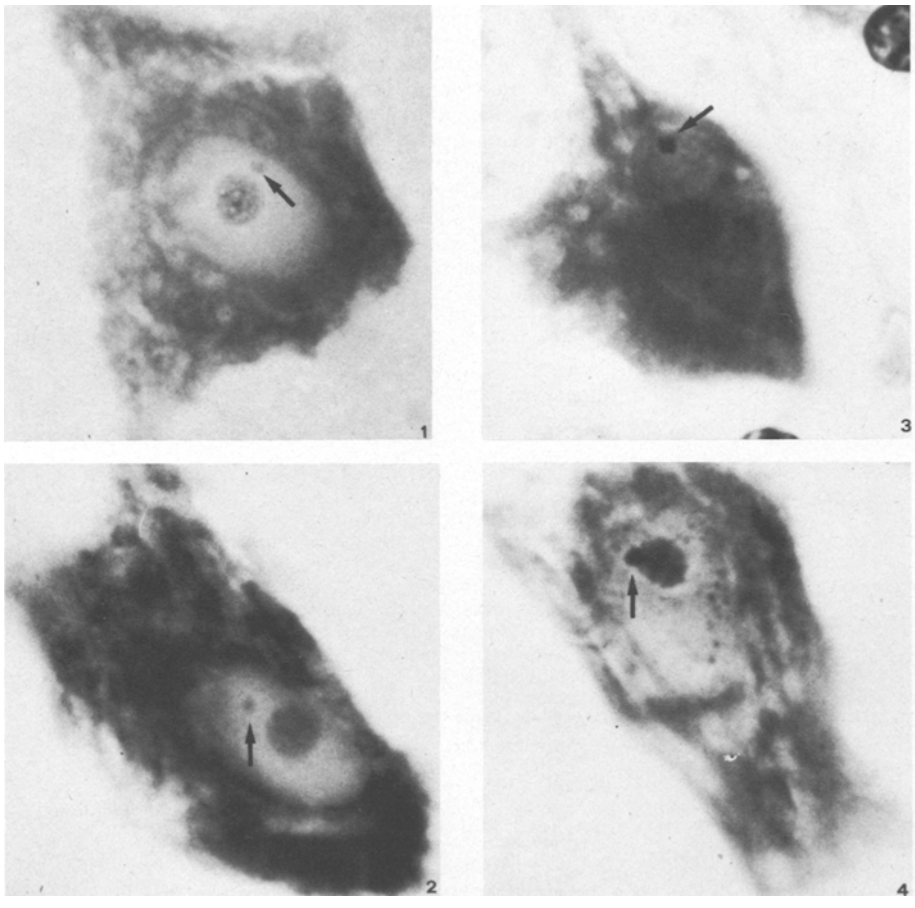

Fig. 1. Photomicrograph of spinal motor neuron of control rat showing clear nucleus, nucleolus and faintly stained Barr body (arrow). Toluidine blue. 1344.

Fig. 2. Photomicrograph of spinal motor neuron of rat, 30 min after reserpine injection. Note diffuse RNA in nucleus, faint Barr body (arrow) and high concentration of RNA in perikaryon. Toluidine blue. 1344.

Fig. 3. Photomicrograph of spinal motor neuron of rat, 4 h after reserpine injection. Note the increase in size and RNA concentration of Barr body (arrow,) which is located near the nuclear membrane, as well as the darkly stained nucleolus and the increase of diffuse RNA in nucleus and cytoplasm. Toluidine blue. 1344.

Fig. 4. Photomicrograph of spinal motor neuron of rat, 24 h after reserpine injection. High concentration of RNA persists in nucleolus and Barr body (arrow), which has moved back to the nucleolus. Concentration of RNA in nucleus and cytoplasm is reduced. Toluidine blue. 1344.

were a further increase in diffuse nuclear RNA and a darkly stained nucleolus. In the majority of neurons fine chromatin granules, dispersed in the nucleoplasm, were also intensely stained. Although the dark staining persisted in the nucleolus and the sex chromatin at 24 h (figure 4), the latter had moved away from the nuclear membrane and was found, in the majority of neurons, near the nucleolus. It was also noted that the concentration of RNA had decreased in the nucleus and cytoplasm.

Discussion. The Barr body in the female represents the inactive second X-chromosome, which in resting cells consists of inert, condensed genetic material[8]. Barr and Bertram[9], who discovered this body, observed that abundant Nissl material appears in the cytoplasm during the recovery phase after intense electrical stimulation of motor neurons; this was correlated with migration of the Barr body to the nuclear membrane. Our findings indicate that at the time of maximal sedation and immobility of the animals the modifications induced by reserpine in motor neurons, i.e. Barr body migration and large increase in cytoplasmic RNA, surprisingly mimic the intracellular changes of the recovery phase described above[9]. However, the main effect of reserpine appears to be the partial euchromatization of the Barr body observed at 30 min with the Feulgen reaction. This decondensation of the sex chromatin precedes the marked increase of RNA observed on this structure at 4 h, which is demonstrated by its intense staining with Toluidine blue. The accumulation of RNA in the perikarya of the motor cells indicates reduced firing activity[10], which is consistent with the immobility of the animals.

Our results indicate that reserpine shares the property of other neuroleptics of interaction with chromatin[6,7,11,12], but with an apparent selectivity for sex chromatin. Since we are interested in the psychoactive mode of action of this drug, the question naturally arises as to the clinical relevance of these experimental findings. Administration of reserpine therapeutically to hypertensive patients very often may cause depression[13]. Depression is an X-linked, genetically transmitted condition with a high preponderance in women[14]. In view of our findings we suggest that the modification in morphology, stainability and localization of the normally inactive second X-chromosome induced by reserpine may indicate functional repercussions related to reactivation of X-linked genes.

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