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Effect of reserpine on the larval-pupal moult of the wax moth *Galleria mellonella* L. (Lepidoptera)[1]

Stanisława Katarzyna Głowacka and A.B. Dutkowski[2]

Electron Microscope Laboratory, M. Nencki Institute of Experimental Biology, 3 Pasteur Str., PL-02-093 Warsaw (Poland), 5 February 1979

Summary. Significant retardation of *G. mellonella* development was induced by reserpine injected to the last instar larvae in doses having a long-lasting effect on the neurosecretory system.

Reserpine, one of the neuroleptic drugs (a tranquillizer), affects many physiological processes in the insect organism. Among other things, it restricts the reproduction of insects. Several investigators described the influence of reserpine on the development and function of female gonads in different species of insects[3-8]. Some authors[3,6] suggested that reserpine acts through the neurosecretory system. This hypothesis was confirmed at the light microscope level in *Tenebrio molitor* and *Tribolium confusum* by Masner[9], and in *Galleria mellonella* by Cymborowski[10], who noted an increased accumulation of neurosecretory material in the neurosecretory cells of pars intercerebralis, induced by reserpine. The drug doses were very low in both cases.

Recent electron microscope studies[11] in *G. mellonella* brain revealed that, after administration of such a low dose of the drug, accumulation of the neurosecretory granules in the perikarya of neurosecretory cells of pars intercerebralis was short-lived. At 24 h after drug administration, the distribution of granules was quite normal. A very pronounced and long-lasting accumulation of neurosecretory material was evoked by a much higher (100×) dose of reserpine (125 µg/g b.w).

The present study was undertaken to see what would be the effect on development of this high dose of the drug. Besides, we wanted to establish the most sensitive age for the drug recipient-insects.

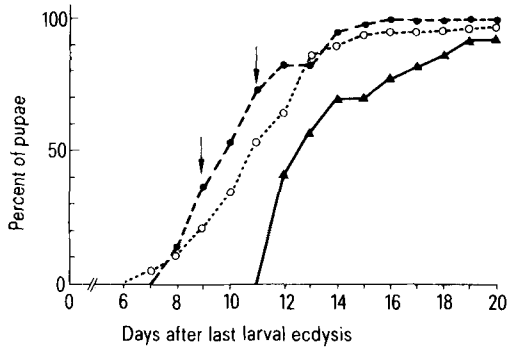
Material and methods. The last (7) instar larvae of *G. mellonella* L. taken from a stock colony cultivated in laboratory conditions (at 30± 1 °C in constant darkness and fed with bee comb) were used in our experiments.

The drug (Serpasil®, Ciba Ltd. in clinical injection vehicle) was injected once, twice or 3 times at 48-h intervals in the high dose of 125 µg/g b.wt into the body cavity of larvae of exactly known age, in a volume of 5 µl. The 1st control group received the same volume (5 µl) of 0.9% NaCl solution (presumed clinical injection vehicle) per specimen. The 2nd control group consisted of uninjected larvae. In our experiments, 24 tranquillized larvae, and 41 injected with saline were analyzed in 5 tests (n = 5) for each group, and 76 untreated larvae in 10 tests (n = 10). The small number of insects in the tranquillized group was due to the lethal properties of this dose of reserpine.

All the larvae were observed daily at 10.00 h for counting the percentage of newly-formed pupae. For comparison, another experimental group was treated as above, but with low doses (1.5 and 3.0 µg/g b.wt) of reserpine. In both cases, the results obtained were evaluated statistically in relation to the control groups. Statistical evaluations were analysed by Smirnov's bilateral test. The homogenity of each group was also tested.

Result and discussion. Injection of small reserpine doses into the body cavity of larvae of the last larval instar of *G. mellonella* produced no effect on the appearance of larval-pupal moult. Neither did a single drug dose of 1.5 µg/g b.wt administered to insects twice (on the 1st and 3rd day of 7th stage) or 3-fold (on the 1st, 3rd and 5th day), nor a single dose of 3.0 µg/g injected once (on the 1st day) cause any apparent differences as compared with control groups.

A high dose (125 µg/g b.wt) of reserpine injected once (on the 1st or 3rd or 5th day of 7th larval instar) caused minimal disturbances in the duration of the last larval stage. Application of this dose on the 3rd day caused a



Percentage of pupae in the group of 3 times tranquillized insects and the control groups in relation to the total number of larvae taken for the experiments. ▲——▲, Tranquillized (n = 5), ●– – –●, control injected with saline (n = 5), and ○·····○, normal (n = 10) insects. Arrows indicate statistically significant differences: ↓, $p < 0.05$, and ↓, $p < 0.001$.

certain prolongation of the last instar; however, it was statistically insignificant.

Contrary to this, a statistically significant delay of pupation was noted only when the drug in the dose of 125 µg/g b.wt was applied 3 times: on the 1st, 3rd and 5th day of the 7th stage (figure 1). Some differences in the percentage of newly-formed pupae were visible on the 9th and 12th day after the last larval ecdysis ($p < 0.05$, figure). On the 9th day, the percentage of new pupae was 0 in treated insects, whereas, in the control group-injected with saline, it reached about 37, and about 21 in untreated insects. On the 12th day, the percentage of pupae increased in all groups, and it attained about 42 in tranquillized insects, about 83 in those injected with saline, and about 64 in normal ones. During the period between the 9th and 12th days, the greatest and statistically most significant retardation was noted ($p < 0.001$, figure).

The percentage of pupae in both control groups permanently increased, attaining 34-54 on the 10th day and 54-73 on the 11th, whereas in the tranquillized group it was 0 all along. In the latter group, the pupation had only started on the 12th day. In both control groups, pupation proceeded parallel. The minimal differences between them were statistically insignificant for the whole period analyzed. These results showed that neither application of saline, nor the injury due to injection, retarded pupation. Almost complete pupation in both control groups was reached around the 16th day. On the contrary, on the same day pupation in the tranquillized group did not even exceed 80%.

Reserpine is retained for a long time in the insect organism[11,12], as well as in other animals[13-15]. As just mentioned, this drug evoked accumulation of neurosecretory granules in the neurosecretory cells of *G. mellonella* brain probably inhibiting, the release of neurosecretory material. Then, 3-fold reserpine administration (on the 1st, 3rd and 5th day) gave a cumulative effect, inhibiting release of neurosecretory material during the whole last larval instar. Therefore, in our experiments pupation started with a certain delay, probably when the neurosecretum had been released in consequence of diminished action of reserpine. Hence, the most effective dose of the drug, applied to larvae of the last stage, was 125 µg/g b.wt when it was injected repeatedly.

It is very interesting to speculate whether such a large drug dose, being sublethal, caused only retardation of larval-pupal ecdysis, or whether it caused some disturbances in other processes of metamorphosis. This problem will be the subject of our next studies.

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Atypical mast cell degranulation and focal hydropic degeneration of venular endothelium in diffuse fibrosing alveolitis

A.W.J. Lykke[1], M.E. Schonell and B.W. Stewart

Schools of Pathology and Medicine, University of New South Wales, P.O. Box 1, Kensington (N.S.W. 2033, Australia), 19 February 1979

Summary. Atypical scroll-like and tubular degranulation of mast cells closely associated with focal endothelial hydropic degeneration is reported in human lung from 4 patients with diffuse fibrosing alveolitis in which the predominant abnormality was hyperplasia and desquamation of type 2 pneumocytes.

Detailed electronmicroscopy study was carried out in 13 patients with diffuse fibrosing alveolitis (diffuse interstitial pulmonary fibrosis). Lung tissue obtained at thoracotomy was fixed immediately with either combined aldehyde or gluteraldehyde fixative. Multiple sections from at least 3 blocks of tissue were examined in each case.

In 8 patients the main finding was moderate or severe interstitial fibrosis with minimal hyperplasia and desquamation of the type 2 pneumocytes. In the remaining 5 patients the predominant abnormality was marked hyperplasia and desquamation of the type 2 pneumocytes with variable fibrosis. In these cases the mast cells were increased in number and in 4 the granules within the mast cells assumed a scroll-like appearance (figure 1). In some sections the granules showed a tubular morphology (figure 1). In the cases with this scroll or tubular type of degranulation, the mast cells were adjacent to areas of focal hydropic degeneration of endothelial cells of venules of the microcirculation (figure 2). Aberrant degranulating mast cells and/or abnormalities of the venular endothelium were not seen in the 8 patients with minimal hyperplasia and desquamation of type 2 pneumocytes.

The association of atypical mast cell degranulation and focal hydropic endothelial degeneration does not appear to have been reported previously in pulmonary diseases nor have we noted this finding in 22 other open lung biopsies. Similar scroll-like mast cell degranulation has previously been described in normal human bronchi and lung[2]. The significance of this type of mast cell degranulation is not known, bit it is important that this ultrastructural change is recognized since it may be confused with viral inclusions, particularly those with morphology similar to rhabdo