

Such a situation is, however, often encountered in a system involving cooperativity, where a fairly large number of identical components may react to form a single complex.

As an illustrative example, the reaction sequence



may be constructed where  $n$  molecules of a protein  $P$  combine successively with components  $S$  and  $C$  in a situation such that  $[C]$  is limited and  $[S]$  is present in excess. Resting level of sodium conductance might then be determined by  $[P_nSC]$ .

For reasonable values of  $n$ , say 5 to 20, the concentration term  $[P_nSC]$  can vary with  $[P]$  in a manner such that it is essentially zero throughout a quite extended range of  $[P]$ , beyond which, it increases abruptly and sigmoidally to approach a limiting value determined by the available  $C$  factor.

Hence it is conceivable that an abrupt reduction in  $[P]$  throughout a narrow range would lead to a gradual time dependent decline in  $[P_nSC]$  which might then be reflected in the  $\bar{V}$  curves of Figures 1 and 2.

While amino acid incorporation into protein of isolated axons is well established for a variety of forms, the synthetic mechanism involved remains obscure<sup>7-12</sup>. The problem is, of course, aggravated by the absence of

clearly demonstrable ribosomes in axoplasm. On separating squid axon sheath from axoplasm, LASEK et al.<sup>13</sup> discovered that most of the ribosomal RNA is confined to the sheath.

Exploration of these results to the medullated axon would implicate the Schwann cell as a source of ribosomal synthesis with subsequent migration of protein into the axon. Not excluded as a possibility is the suggestion by AUSTIN and MORGAN<sup>14</sup> that since high levels of RNA are found in synaptosomal structures it is possible that the axonal membrane itself may contain ribosomal types of synthesizing systems.

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## Alpha-Receptor Subsensitivity of Isolated Atria from Rats Following Repeated Injections of Phenylephrine or Isoprenaline<sup>1</sup>

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**Summary.** Daily injections of phenylephrine or isoprenaline for 4 or 7 days lowered the chronotropic sensitivity of  $\alpha$ -adrenoreceptors in isolated rat atria.

An increased sensitivity of cardiac  $\alpha$ -receptors is found to be induced in frogs and/or mammals by several conditions, e.g. by low experimental temperature<sup>3-7</sup>, inactivity of skeletal muscles<sup>8</sup>, increased vagal influences on heart<sup>9</sup>, propylthiouracyl treatment<sup>10,11</sup>, and hypothyroidism<sup>12</sup>. These effects are usually associated with a lowered  $\beta$ -receptor sensitivity shown in inotropic and/or metabolic activity of this organ. Recently we<sup>13</sup> found out that cold acclimation temporarily lowers chronotropic sensitivity of the isolated rat atria to an  $\alpha$ -adrenergic drug phenylephrine (PHE), while it does not change the sensitivity to a  $\beta$ -adrenergic drug isoprenaline (ISO). These results were interpreted by suggesting that the enhanced release of catecholamines, as a result of increased sympathetic activity of cold-exposed animals, was responsible for this subsensitization of cardiac  $\alpha$ -receptors.

The aim of the present study was to elucidate whether increased levels of  $\alpha$ -stimulant (PHE) and  $\beta$ -stimulant (ISO) in rats, as produced by repeated injections of these drugs, will change the sensitivity of cardiac adrenoreceptors in isolated atria.

**Material and methods.** A total of 198 adult male Sprague-Dawley rats, 200–350 g in weight, were used in these studies. They were divided into 20 groups, half of them receiving once a day subcutaneously injections of PHE (Neosynephrine®, Winthrop) 3 mg/kg in physiological NaCl-solution or ISO (Isuprel®, Winthrop) 0.3 mg/kg in

olive oil. The duration of the treatments in each group is mentioned in the Figures. The other half served as control animals receiving injections of NaCl-solution or olive oil without any drugs.

The cumulative concentration-response curves for the chronotropic response to ISO and PHE were determined on isolated atria at 37°C in Thyrode's solution. The

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contractions were recorded by means of a suction electrode on a Mingograph 24 B jet recorder as described earlier<sup>13</sup>. A single concentration-response relationship curve was determined with each atrial preparation.

**Results.** The graphs in Figure 1 show that repeated PHE-injections did not cause any significant changes in atrial response to ISO, but they had lowered the positive chronotropic sensitivity to PHE after 4 days treatment. This can be seen in the concentration-response curve shifting to the right. Because this treatment strongly reduced the maximum response ( $p < 0.01$ ), the  $EC_{50}$ -values, however, did not differ from each other in control and experimental groups. This lowered sensitivity was partly returned to normal after 7 days and totally returned after 25 days of injection treatment.

The results in Figure 2 indicate the effects of repeated ISO-injections on adrenergic atrial response and were very similar to that of chronic PHE-treatment. The response to ISO was not significantly changed but the concentration-response curves to PHE were shifted to the right indicating a lowered sensitivity of  $\alpha$ -receptors following chronic treatment of 7 and 20 days with ISO. The maximum responses were reduced to about 50% after 7 days and to 60% after 20 days of injection treatment as compared to the control values and statis-

tical significancies were  $p < 0.01$  and  $p < 0.005$ , respectively. In the 20-day treatment groups, all responses were exceptionally great. This is probably due to seasonal differences in rats, for these experiments were carried out about 6 months later than the other experiments.

The basic contraction frequencies of isolated atria were not changed significantly in any of the PHE-treated rats, but they were lowered in ISO-treated animals. After 20 days of the ISO-injection treatment, the mean value of the ISO-treated group was  $184 \pm 8.0$  and that of the control group was  $219 \pm 8.8$ ;  $p < 0.025$ .

**Discussion.** The changes in atrial responses found in the present study are quite similar to those observed by HARRI et al<sup>13</sup> in isolated atria from cold-exposed rats.

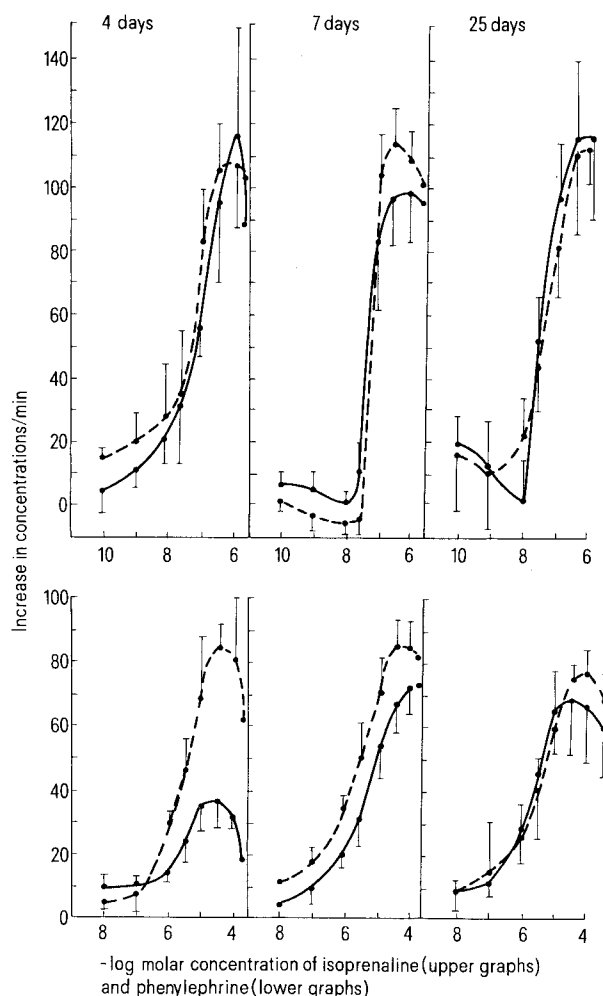


Fig. 1. Log concentration-response curves for the chronotropic responses to isoprenaline and phenylephrine in atria from control rats (broken lines) and from animals injected with phenylephrine for 4, 7 or 25 days (continuous lines). Vertical bars indicate  $\pm$  or  $-$  SE.

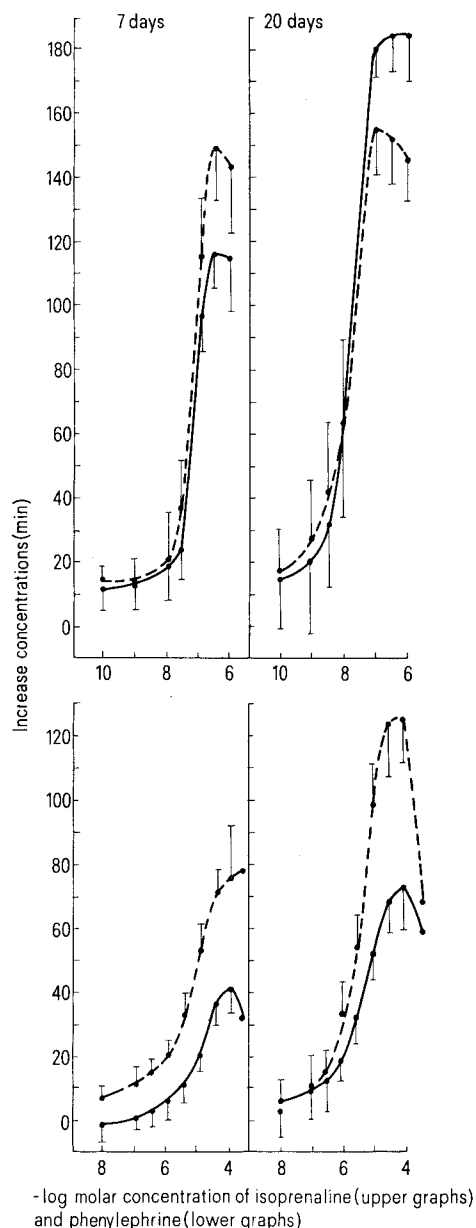


Fig. 2. Log concentrations-response curves for the chronotropic responses to isoprenaline and phenylephrine in atria from control rats (broken lines) and from animals injected with isoprenaline for 7 or 20 days (continuous lines). Vertical bars indicate  $\pm$  or  $-$  SE.

The lowered sensitivity to PHE but not to ISO was found in both studies. Only a prolonged cold-acclimation induced an increased response to ISO, but by then the responses to PHE had already returned to their initial levels<sup>13</sup>. LEBLANC et al.<sup>14</sup> have observed an increased  $\beta$ -receptor sensitivity by repeated injections of ISO and by cold-acclimation of 20 days in rats. This was found in the metabolism of the animals but also in cardiac chronotropic response. However, these results are not quite comparable with those in the present study because their experiments were carried out in vivo, where reflex adjustments and other secondary influences, e.g. an increased metabolism in the animals, are involved.

KUNOS et al.<sup>5</sup> have suggested that cardiac  $\alpha$ - and  $\beta$ -receptors represent allosteric conformations of the same structure. According to their findings that hypothyroidism reduces the  $\beta$ -receptor sensitivity and raises the  $\alpha$ -receptor sensitivity, they have further suggested that it is an altered thyroid hormone level which results in an interconversion of myocardial  $\alpha$ - and  $\beta$ -receptors<sup>12</sup>. Our present and previous results on cold-acclimated rats<sup>13</sup> are not totally in agreement with the ideas of KUNOS et al. mentioned above. The lowered  $\alpha$ -receptor sensitivity was not associated with a simultaneous increase in  $\beta$ -receptor response, thus indicating no interconversion of adrenoceptors. BENFEY<sup>15</sup> also has recently reported an opposing suggestion to the adrenoceptor transformation hypothesis. HARRI et al.<sup>13</sup> suggested that an increase in the sympathetic activity of cold-exposed rats was responsible for the subsensitivity of cardiac  $\alpha$ -receptors.

The present results from rats with higher sympathomimetic amine levels produced by repeated injections of sympathomimetic amines support this suggestion. In agreement are also the findings that the decreased sympathetic activity in rats, resulting from decentralization or from 6-hydroxydopamine treatment, causes supersensitivity to noradrenaline but not to ISO<sup>16,17</sup>. However, the role of thyroxine cannot be excluded. It has been shown that cold-exposure<sup>18</sup> and  $\beta$ -adrenergic stimulation by ISO<sup>19</sup> induce thyroid hormone secretion. Thus a change in sympathetic activity reflects changes in thyroid hormone levels<sup>20</sup>, and this could induce changes in cardiac adrenoceptors. Negative results, however, are also reported of the effects of thyroxine on the cardiac adrenoceptor sensitivity<sup>21,22</sup>.

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## Occurrence of Aldehyde-Fuchsin and Performic Acid-Victoria blue Positive Granules in the Ovarian Pedicle of *Dysdercus koenigii* F. (Pyrrhocridae: Heteroptera)

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**Summary.** A cycle of activity of aldehyde-fuchsin and performic acid-Victoria blue positive granules was observed in the ovarian pedicle of *Dysdercus koenigii* during the first ovipositional cycle. The quantitative variation of these granules in the pedicle can also be correlated directly with the increase or decrease of the neurosecretory material in the A-type cells of the pars intercerebralis medialis region of the protocerebrum of the brain.

The occurrence of Gomori-positive granules in the amoebocytes was earlier reported in *Rhodnius*<sup>2</sup>. Thereafter, the chrome haematoxylin-phloxin (CHP), aldehyde-fuchsin (AF) and performic acid-alcian blue (PAAB) positive granules were observed in the last vitellarial follicles of *Pyrilla perpusilla*<sup>3</sup>. During the course of investigation on the endocrine control of oocyte maturation, a cycle of activity of AF and performic acid-victoria blue (PAVB) positive granules were observed in the ovarian pedicle of *Dysdercus koenigii*. The present study presents some histological and histochemical observations of these AF and PAVB positive granules in the ovarian pedicle and a discussion of their possible functional significance.

The test-insects (*D. koenigii*) were reared in the laboratory at a temperature of  $28^{\circ} \pm 2^{\circ}\text{C}$  and watersoaked cotton seeds were provided as food. For the purposes of the present study, a requisite number (8–9) of dated female adults were dissected daily in physiological saline<sup>4</sup> for the whole period of one ovipositional cycle of 8 days. The neuroendocrine organs and the gonads from the same individuals were fixed in appropriate fixative and were

then stained with AF and PAVB in the manner described by DOGRA and TANDAN<sup>5</sup>. The preparations were supplemented by histological sections stained either before (PAVB) or routinely after sectioning (AF).

An examination of preparations (both whole mounts and histological sections) of the neurosecretory cells and gonads (ovarian pedicle) revealed a quantitative variation of AF and PAVB positive granules, during the different stages of egg ripening. The staining reaction of these pedicle granules and those of A-type cells were identical. Moreover, the quantitative variations of these granules in the pedicle can also be correlated directly with the increase

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