potential (A–C), showing that there was little or no inhibitory action. However, after spinalization the usual large inhibitory postsynaptic potential appeared in records F and G. On the other hand the inhibitory potential caused by Ib impulses from the lateral gastrocnemius-soleus nerve appeared before as well as after section of the cord.

It has been assumed that the functions of the Ib systems in the reflex taxis of the animal would be protective, i.e. too strong a contraction of the muscle would be prevented by autogenetic inhibition[7]. However, the pattern of connections in the spinal animal suggests an additional function of the Ib action. The large inhibitory action exerted by Ib impulses from flexor digitorum longus would appear with the active plantar flexion of the toes, at the height of the extension phase of the step and could be of importance for terminating the extension phase and initiating the flexion phase of stepping.

If impulses from flexor digitorum longus normally were a contributor in the reflex regulation of stepping, these inhibitory actions would clearly require control from higher centres, for example during voluntary contraction of the flexor digitorum longus or during running. A control at the interneuronal level would certainly be most effective. Such an inhibitory control presumably exists, although less pronounced, for Ib inhibitory actions from other muscles than flexor digitorum longus.

The functional implication of the suprasegmental control of interneurones mediating group II and III impulses may be a different one. Attention was drawn above to the co-existence in the spinal preparation of the entirely different pattern of synaptic actions caused by the two types of muscle spindle afferents, groups Ia and II respectively. In the present experiment there was no evidence that the synaptic linkage between Ia fibres and motoneurones was controlled from higher centres. On the other hand, with the interneurones mediating the group II and III actions, there exist very effective inhibitory control mechanisms from supraspinal centres. It may tentatively be suggested that in normal locomotion there is assistance by reflex actions from the Ia and Ib systems, but on account of this inhibition at the internuncial level impulses in group II and III fibres are largely without synaptic actions on motoneurones.

Zusammenfassung

An decerebrierten und spinalen Katzen wurden diverse somatische Afferenzen in ihren synaptischen Wirkungen auf Vorderhornzellen verglichen. Bei der decerebrierten Katze waren deren erregende und hemmende Wirkungen stark herabgesetzt oder nicht nachweisbar. Somit werden Zwischenneurone im Reflexbogen durch supraspinale Zentren tonisch gehemmt. Die Bedeutung dieser Kontrolle für die Reflexregulierung wird besprochen.

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Brèves communications - Kurze Mitteilungen Brevi comunicazioni - Brief Reports

Selective Depletion of Nor-Adrenaline in the Adrenal Medulla of the Rat After Administration of Reserpine (Histochemical Research)

Recent research (CARLSSON and HILLARP[1], KRONEBERG and SCHÜMANN[2], MOLINATTI *et al*[3]) has demonstrated that the alkaloids of '*Rauwolfia serpentina*' cause the liberation of catechol amines by the adrenal medulla of animals of different species.

In the rat, the administration of reserpine causes a definite modification of cellular chromaffinity; alongside various groups of cells completely devoid of pheochrome substance, the remaining glandular parenchyma preserves a normal degree of chromaffinity (MOLINATTI *et al.*[3]). As demonstrated by HILLARP *et al.*[4] and ERÄNKÖ[5], adrenaline and nor-adrenaline in the rat's medulla are contained in two distinct types of cells. Therefore the particular behaviour observed in this animal has suggested to us that under the action of reserpine only one of the two cathechol amines is selectively secreted.

This investigation therefore was carried out in order to examine the possible differences in behaviour of adrenaline and nor-adrenaline. The latter may be demonstrated histologically by means of HILLARP-HÖKFELT's reaction[6], which consists in the formation of a pigment insoluble in

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[2] G. KRONEBERG and H. J. SCHÜMANN, Arzneimittelforschung *4*, 279 (1957).

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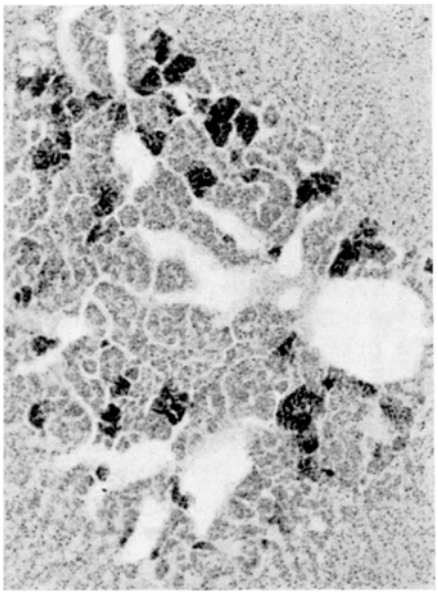
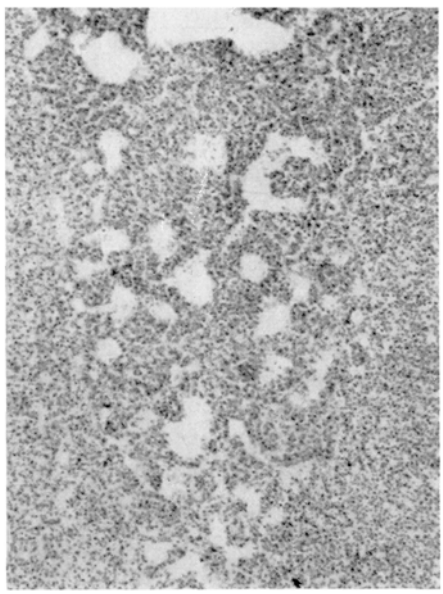
[6] N. A. HILLARP and B. HÖKFELT, J. Histochem. Cytochem. *3*, 1 (1955).



A



B

Fig. 1. – Adrenal medulla of rat before and after treatment with reserpine. Hillarp-Hökfelt's reaction. In *A*, the cellular groups appear containing a dark yellow pigment which disappears entirely after administration of reserpine (*B*). 65×.

water due to the oxidation of nor-adrenaline with potassium iodate; whereas oxidation with chromic salts enables the two catecholamines to be demonstrated simultaneously.

Methods.—20 albino rats, Wistar stock, male sex, average weight 200 g were used. The animals were divided into two groups and treated with reserpine, in doses of 0·5 mg and 1 mg/kg body weight respectively. The injections were made endoperitoneally and were repeated for 3 consecutive days to the animals of the first group and for 5 days to those of the second group. A control group was given injections of physiological solution in the same way.

At the end of the treatment all the animals were killed by decapitation. The adrenals glands were immediately removed and cut into thin sections; some were immersed for 48 h in a 10% solution of potassium iodate (HILLARP's specific reactive for nor-adrenaline) and then, after fixation in formalin, cut with the freezing microtome; others were immersed for 3 days in a solution of chromic salts

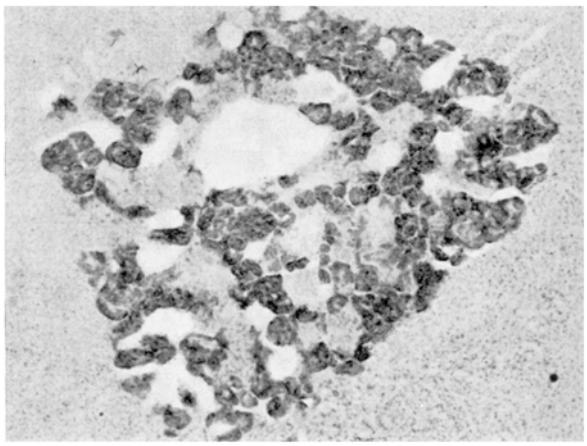
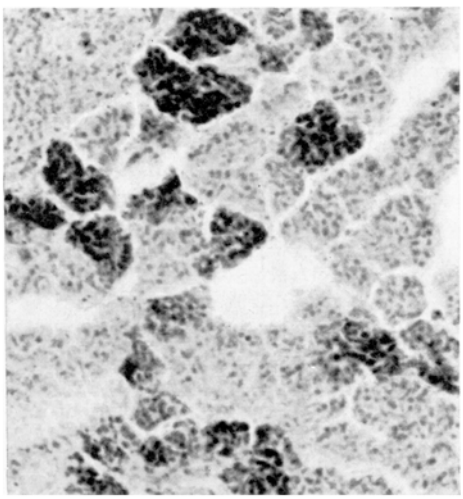
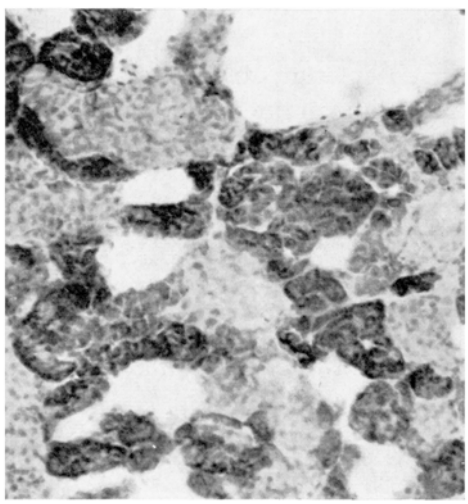


Fig. 2. – Adrenal medulla of rat. Chromaffin reaction. After administration of reserpine cellular islets completely free from pheochrome granules are encountered. 65×.



C



D

Fig. 3. – Detail of Figure 1*A* (*C*) and Figure 2 (*D*). 130×.

(10 parts of 5% potassium bichromate + 1 part of 5% potassium chromate) and afterwards enclosed in paraffin.

Results.—In the control group the sections of adrenal medulla treated with chromic salts showed a normal degree of chromaffinity in all the cells, while in those treated with HILLARP's reagent cellular groups were evident containing a dark yellow pigment and irregularly distributed in the remaining parenchyma, which was completely devoid of pigment (Fig. 1*A*, 3*C*). In each instance the adrenals of the treated rats showed a histochemical picture strikingly different from that of the normal gland. When doses of 0·5 mg/kg were used , with the chromaffin reaction cellular islets could be observed in the adrenal medulla completely free from pheochrome granules (Fig. 2, 3*D*). These cellular groups were irregularly distributed; the number of their cellular elements was quite variable. However most of the parenchyma preserved a normal degree of chromaffinity. A sudden passage, without intermediary stages, was always observed between the groups of non-chromaffin cells and the adjacent cellular columns, which were intensely pheochrome. With the HILLARP's reaction potassium iodate, however, it was never possible to show, in any cell, the formation of the characteristic dark pigment (Fig. 1*B*). On the contrary, the administration of reserpine in stronger doses (1 mg/kg for 4–5 consecutive days) caused the almost complete disappearance of the chromaffinity of every glandular element; the cellular cytoplasm appeared more or less vacuolised, relatively basophil, and completely devoid of pheochrome granules. With intermediate doses increasing progressively from 0·5 mg to 1 mg/kg, a progressive reduction was noted in the number of cells positive to chromaffin reaction; in all these stages HILLARP's reaction was consistently negative.

Discussion.—In agreement with what has been demonstrated by other authors by chemical methods (CARLSSON and HILLARP[1], KRONEBERG and SCHÜMANN[2]) and by our previous research[3], it may be considered, from examination of the histochemical findings described, that the administration of reserpine causes a more or less intense diminution of the catechol amine content in the adrenal medulla. The size of this discharge seems proportionate to the dose of alkaloid administered, to the point of reaching with the highest dosage, the complete depletion of the catecholic content of the gland.

The finding of total disappearance of dark yellow pigment after oxidation with potassium iodate in the cellular tissue of the rats treated with reserpine in doses of 0·5 mg/kg denotes a complete depletion of nor-adrenaline; and since, under these conditions, the chromaffin reaction still demonstrates a high number of positive cells, it may be deduced that the adrenaline content has not, on the contrary, undergone any notable modifications. On the other hand, by comparing of the sections treated with chromic salts with those subjected to oxidation with potassium iodate, it can be observed that the number and the topografical distribution of cellular elements devoid of pheochrome material correspond fairly well the number and the distribution of the cellular elements which, in the normal gland, seem to be rich in pigment with HILLARP's reaction.

Therefore it can be stated that, in the above-considered doses and within the limitations imposed by the histochemical reactions adopted, reserpine causes an almost selective discharge of nor-adrenaline; with stronger doses, besides the liberation of nor-adrenaline, there seems to be also a progressive diminution of adrenaline up to the complete disappearance of all the catecholic content.

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Institute of Medical Pathology of the University of Turin (Italy), March 2, 1958.

Riassunto

Con tecniche istochimiche è stata indagata l'azione della reserpina sul contenuto catecolico della midollare surrenale del ratto. Viene documentata una scarica pressochè selettiva di nor-adrenalina.

Histochemische Untersuchungen über den Einfluss von Iproniazid (Marsilid) auf die durch Reserpin erzeugte Freisetzung von Adrenalin und Noradrenalin aus dem Nebennierenmark

Chemische und histochemische Untersuchungen ergaben, dass Iproniazid durch Hemmung der Monoaminoxydase (MO) *in vivo* nicht nur den Abbau von «freiem» 5-Hydroxytryptamin (5HT, Serotonin) verhindert[1], sondern möglicherweise auch die nach Injektion von Reserpin feststellbare Freisetzung des gespeicherten 5HT aus den Körperdepots hemmt[2]. Grosse Dosen Reserpin setzen auch den Katecholamingehalt verschiedener Gewebe[3] und des Blutplasmas[4] herab, eine Wirkung, die im Gehirn durch Iproniazid-Vorbehandlung signifikant abgeschwächt wird[5]. In der vorliegenden Arbeit soll mit histochemischen Methoden geprüft werden, ob eine solche Abschwächung der Reserpinwirkung an den Katecholaminen des Nebennierenmarkes der Ratte sichtbar gemacht werden kann.

Versuchsanordnung. Gruppen von 8 bis 12 männlichen oder weiblichen, 80 bis 120 g schweren Wistarratten eigener Zucht wurden wie folgt behandelt (Tabelle I):

Iproniazid wurde als Phosphat (Marsilid*), Isoniazid als Base (Rimifon*) in äquimolarer Dosierung verwendet. 24 h nach der Reserpininjektion wurden die Ratten getötet (Kontrollgruppen VIII und IX gleichzeitig mit Gruppen VI und VII), die Nebennieren herauspräpariert, einmal zerschnitten und lebensfrisch fixiert. Der *histochemische Nachweis der Katecholamine* erfolgte nach folgenden Methoden: Gruppen I bis V: Fixation in Müllerscher Lösung (Kaliumbichromat 2,5 – Natriumbisulfit 1,0 – Aqua dest. *ad* 100,0) während 3 Tagen, Paraffineinbettung und Gegenfärbung mit Haemalaun.

Gruppen VI bis IX (nach der Vorschrift von HILLARP und HÖKFELT[6]): Eine Nebenniere pro Tier, während 24 h

* Fabrikmarke.

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