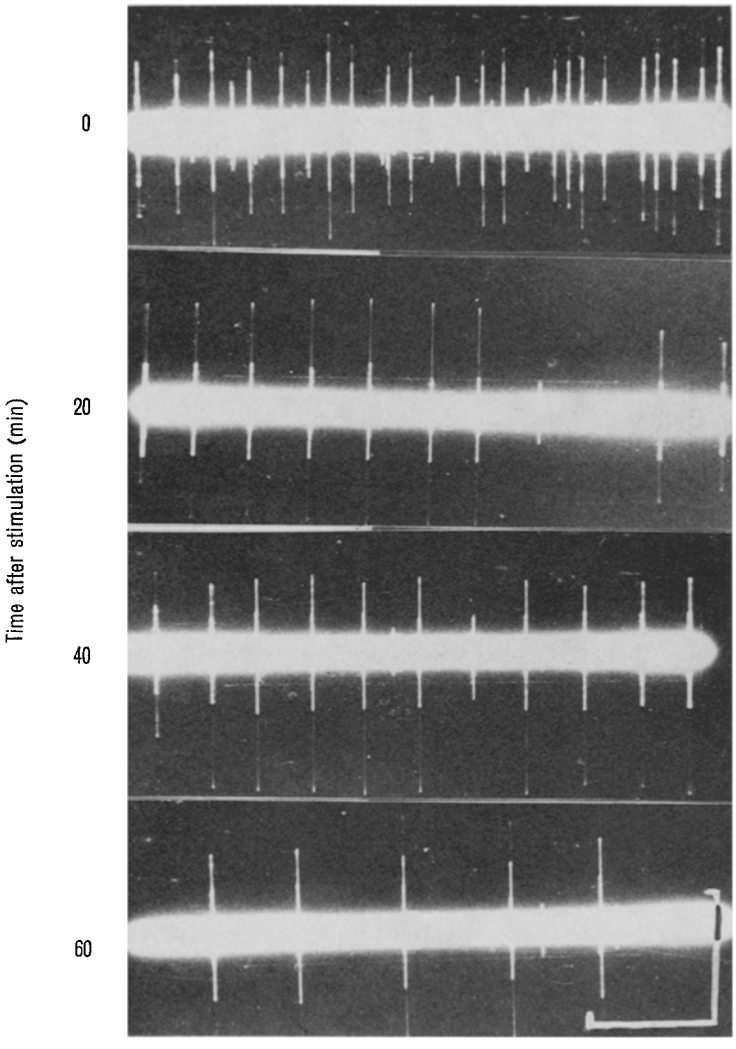

Fig. 2. The effect of electrical stimulation of the paraventricular nucleus (PV) on the multi-unit discharge rate of the controlateral PV. Time marking 0.1 sec; calibration 100 μV.

injection of oxytocin and vasopressin[11], it is reasonable to consider a relation between the changes in neuronal activity occurring after stimulation of diencephalic structures and a release of oxytocin and vasopressin into the CSF, as one possible explanation of the findings. According to our experience, this is suggested by the relatively long latency prior to the occurrence of initial and/or maximum effects, for it is also after intraventricular injection of oxytocin and vasopressin that comparable effects were found[11]. The present findings appear to support the assumption that after stimulation of PV and SO, oyxtocin and vasopressin, respectively, are discharged into the CSF. Thus, a modulating influence on the entire central nervous system through the neurohormones contained in the CSF would be most likely because of their half-life of approx. 30 min.

Zusammenfassung. Elektrische Stimulation neurosekretorischer Kerngebiete des Zwischenhirns führt zu charakteristischen Veränderungen der neuronalen Aktivität angrenzender hypothalamischer Neuronenpopulationen, die einen modulierenden Einfluss von Neurohormonen auf das ZNS vermuten lassen.

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Changes in DBH and Noradrenaline Resulting from Cold Exposure

Dopamine β-hydroxylase (DBH) is the most reliable marker for noradrenaline storage vesicles: it has been shown to be associated with both large and small terminal vesicles[1] as well as the large axonal vesicles[2,3]. In axonal vesicles 18% of the DBH is soluble and releasable by hypo-osmotic shock[3]; the percentage of soluble DBH in small terminal vesicles, the main source of released transmitter[4,5], is not known; it is likely to be less than in the large noradrenaline storage vesicles since the volume to surface ratio decreases steeply with vesicle diameter.

The finding that sympathetic nerve stimulation leads to the appearance of soluble DBH in the perfusate lends support to the hypothesis that noradrenaline is released by exocytosis[6]. However, nothing is known of the sub-

sequent fate of the empty noradrenaline storage vesicle. Estimates of the vesicle life span range from 4 weeks[7] to 40 h[8]. The former figure implies freqeuent refilling and re-use of the vesicles, the latter is compatible with a single cycle of filling and emptying and non-re-use of vesicles. Experiments on the adrenal medulla[9] suggest that chromaffin granules cannot refill with catecholamines after they have released their contents.

In the present experiments changes in DBH activity and noradrenaline content of nerve terminals resulting from transmitter release were measured. Rats were exposed to 3 °C for periods ranging from 5–30 min. They were killed by a blow on the head, the heart ventricles removed and homogenised in groups of 3. The vesicular pellet was isolated by differential centrifugation and after resuspension assayed for DBH activity, noradrenaline and protein content (Fillenz and West, in preparation). The vesicular noradrenaline shows a rise at 5 min after which it returns to control levels (Figure). The DBH activity on the other hand is reduced as a result of cold exposure. Table I shows the mean noradrenaline and DBH levels over the period from 10–30 min compared to simultaneous control values. The DBH is reduced by 32%. If this depletion were to represent loss of soluble DBH resulting from exocytosis, it would imply the release of almost twice the total vesicular store. Since this is unlikely, it suggests that some of the vesicles may be lost or destroyed after release. The only report so far of a reduction in the DBH activity of nerve terminals is that of BRIMIJOIN et al.[10], where a reduction of 12% in the DBH activity of the rat heart was found 4 h after the administration of reserpine.

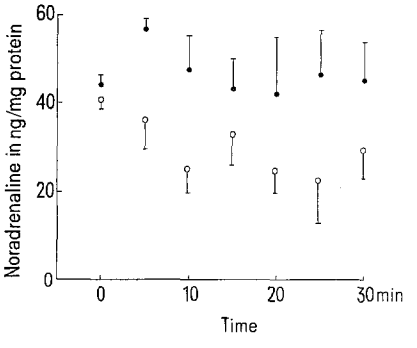
Table I. Changes in mean DBH activity (DBH), noradrenaline content (NA) and noradrenaline to DBH ratio (NA:DBH) of rat heart vesicular fraction, relative to simultaneous control values, resulting from cold exposure for 10–30 min (see Figure)

	DBH	NA	NA:DBH
Cold/Control	0.68 ± 0.06^{b} $n = 15$	1.11 ± 0.09 $n = 19$	1.56 ± 0.19^{a} $n = 14$

DBH expressed in ng of phenylethanolamine/30 min incubation/mg protein; noradrenaline expressed in ng NA/mg protein; NA:DBH ratio is the mean of individual values compared to simultaneous control values. Mean ± S.E.M. [a] $P = 0.01$, [b] $P = 0.001$.

Table II. Mean DBH activity (DBH), noradrenaline content (NA) and noradrenaline to DBH ratio (NA:DBH) of rat heart vesicular fraction from control animals and animals given 150 µg/kg l-noradrenaline intravenously (Exog. NA)

	Control	Exog. NA
DBH	40.6 ± 2.2 $n = 32$	35.7 ± 5.9 $n = 10$
NA	43.9 ± 2.4 $n = 31$	72.5 ± 5.9^{b} $n = 10$
NA:DBH	1.19 ± 0.10 $n = 28$	2.12 ± 0.15^{a} $n = 10$



Changes in DBH activity and noradrenaline content of the vesicular fraction of rat heart. ●, Noradrenaline content; ○, DBH content; C, control value; Abscissa: time in min during which rats were exposed to 3 °C. Ordinate: noradrenaline in ng/mg protein; DBH in ng phenylethanolamine formed/30 min incubation/mg protein. Each figure is the mean ± S.E.M.

The rise in vesicular noradrenaline suggests a rapid increase in synthesis rate. An increase in vesicular noradrenaline occurring at the same time as a loss of vesicles could be explained if the remaining vesicles could increase their noradrenaline content. That the noradrenaline storage capacity of vesicles is normally not fully saturated was demonstrated by the increase in vesicular noradrenaline produced by the administration of exogenous noradrenaline or the inhibition of monoamine oxidase[11]. Table II shows the DBH and noradrenaline concentrations of vesicular fractions of rat heart from control animals and animals given 150 µg of noradrenaline/kg body weight. We have used the noradrenaline to DBH ratio (NA:DBH ratio) as a measure of the degree of saturation of the noradrenaline storage capacity. (see Table II). Table I shows the NA:DBH ratio of cold treated rats compared to simultaneous control values. It will be seen that transmitter release causes the NA:DBH ratio to rise although it does not reach the level obtained after administration of exogenous noradrenaline.

These results suggest that the supply of vesicles as well as the rate of transmitter synthesis may be factors which limit and control the storage and release of transmitter[12,13].

Résumé. L'exposition au froid, pour des périodes de 30 min, entraîne une réduction de 32% de l'activité du DBH de la fraction vésiculaire du ventricule du cœur du rat. Le contenu en noradrénaline, après une augmentation brève, reprend sa valeur normale. Il est suggéré que la libération accrue provoque une diminution du nombre des vésicules et un remplissage accru des vésicules restantes.

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