content of blood after bleeding[15,16], the doses of vasopressine employed in our experiments will be within the physiological limits.

After bleeding, a significant decrease of the blood flow in the skin has been observed by SAPIRSTEIN et al.[17] and by TAKÁCS et al.[18] in the rat. Possibly this phenomenon may be caused by a release of vasopressine. This hypothesis is supported by two observations: a significant increase of the vasopressine level in the blood has been observed after bleeding[15,16]; on the other hand, dogs tolerate bleeding less well after neurohypophysectomy[19].

Zusammenfassung. Vasopressin in den Blutdruck nicht erhöhenden geringen Dosen (0.01 E/kg i.p.) verringert die Hautdurchblutung der Ratte und erhöht die zirkulatorische Resistenz. In höheren Dosen von sogar 1.0, 0.1 E/kg beeinflusst Vasopressin Minutenvolumen und Zirkulation anderer Organe nicht.

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The Neurogenic Activity of High Potency Substance P

There appears to be general agreement that 'crude' substance P (SP) has an action on the nervous system[1–6]. However, there appears to be some disagreement as to whether the neurotropic action is retained with purification. Thus, STERN and HUKOVIĆ[7] have reported that as SP is purified up to 270 U/mg, it retains its ability to antagonize morphine-induced analgesia, but looses its ability to produce tranquilization. On the other hand, HAEFELY and HÜRLIMANN[8] have stated that 'purified' SP is without neurotropic activity.

This paper relates research undertaken in an attempt to determine whether the neurotropic activity of SP is lost when the material is purified to a potency of 10,000 U/mg.

Methods and Materials. In order to provide the most efficient utilization of the small amounts of SP available, the test system used was that previously described from these laboratories[6], i.e. potentiation of the fourth dorsal root potential (DR IV), by substance P in the presence of lysergic acid diethylamide (LSD).

Decerebrate cats were used in all of these experiments. Decerebration and laminectomy were performed under ether anesthesia. Dorsal root potentials (L7 or S1) were evoked at a frequency of 2.5 cps, using stimuli approximately 50% of maximal and 0.05 msec duration. Ether anesthesia was stopped at least 1 h prior to the administration of SP or LSD. No experiment was initiated until the dorsal root potentials were observed to be constant for at least 30 min.

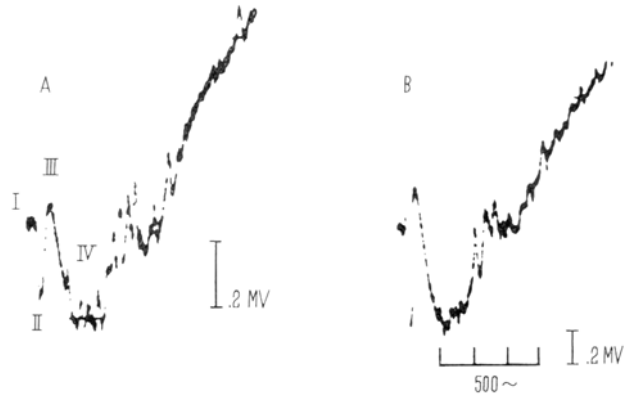
After a control period of 30 min, LSD, 20 μg/kg, was injected i.v. After a period of approximately 10 min, during which time DR IV was seen to increase in amplitude, and then to remain constant at the new level, SP was injected i.v.

The SP used in these experiments was of three potencies, 10 U/mg, 1,000 U/mg, and 10,000 U/mg[9], and was purified as described earlier[10].

Results. (1) LSD: In each instance the administration of LSD was followed by an increase in DR IV. This had been described earlier[6], and no further comment is necessary at this time.

(2) SP: The results of administration of each of the three samples of SP can be described together since the results were qualitatively similar. In each instance SP, after LSD, produced a further augmentation of DR IV. In addition, in certain instances (Figure) this was associated with an increase of the first three dorsal root potentials (i.e. DR I, II, and III). Augmentation of DR I, II, and III with larger doses of SP and presumedly consequent to augmentation of DR IV has been observed and discussed in earlier reports[6].

Using SP of a potency of 10 U/mg, augmentation was seen in three cats using doses ranging from 20–40 U/kg. Using SP of a potency of 1,000 U/mg, the phenomenon



Sample traces of records from the same experiment illustrating the actions of SP (10,000 U/mg) on the dorsal root potential of the cat. Control record (A), illustrates a typical dorsal root potential (DR I–IV) after the administration of LSD, 20 μg/kg. 6 min prior to trace B, SP, 20 U/kg, was injected. Note the change in calibration of the vertical amplifier.

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was observed in four cats, the dose range being 30–40 U/kg. Using SP 10,000 U/mg, the same phenomenon was seen in six cats, the dose range being from 20–70 U/kg. Where possible, the duration of these actions of SP was followed and found to be in excess of 30 min. No attempt was made to determine the minimal effective dose.

Discussion. These experiments were undertaken for the express purpose of determining if highly 'purified' SP has neurotropic activity, and with the ultimate goal of determining whether or not this activity is lost during purification of the leiotropic fractions, thereby explaining the discrepancy between STERN and HUKOVIĆ[7] and HAEFELI and HÜRLIMANN[8]. However, the data presented here show that SP, of relatively high potency, still contains neurotropic activity. Unfortunately, the nature of the test preparation used precludes quantitation of the information obtained, and it is not possible to suggest whether the leiotropic and neurotropic actions are due to the same component.

These data are in support of the conclusions of STERN and HUKOVIĆ[7] that purified SP has neurotropic activity[11].

Zusammenfassung. Gereinigte Präparate von Substanz P mit Aktivitäten von 10, 1000 und 10000 E/mg verstärken das vierte Potential der dorsalen Nervenwurzel im Rückenmark der Katze (Verabreichung nach LSD). Unsere Befunde bestätigen frühere Beobachtungen und legen die Vermutung nahe, dass bei fortschreitender Reinigung ein Teil der neurotropen Aktivität zusammen mit der leiotropen angereichert wird.

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PRO EXPERIMENTIS

Microdetermination of Magnesium and Calcium in Animal Tissue

In connection with studying the metabolism of kidney cortex slices, the necessity arose to determine microgram quantities of Mg and Ca in this material. None of the known methods can satisfactorily remove all the difficulties associated with such determination, which necessitates complete separation of Mg and Ca from each other, and of the two from phosphate and from interfering heavy metals, in particular Fe.

The method presented here is based on the separation of metals on ion exchanger[1]. The technique of purifying the resin and the effect of cross-linkage on the strength of the bond of the two metals to the resin was assessed.

Experimental. Reagents: The water used was redistilled from a quartz apparatus. The solutions were kept in polyethylene bottles.

$5 \times 10^{-3} M$ EDTA.

Buffer, pH 10.5, $1M$ NH_4Cl–NH_4OH.

Buffer, pH 5.6 (20 ml $1N$ CH_3COOH + 180 ml $1N$ CH_3COONa).

0.06% methanolic solution of Eriochrome Black T or Calcon.

0.1% ethanolic solution of 8-hydroxyquinoline.

0.018% aqueous solution of sodium naphthalohydroxamate[2].

Dowex 50-X8 (200–400 mesh).

All chemicals were of analytical purity.

Method. The method was tested on standard solutions and applied to the determination of Mg and Ca in the ash of rabbit and rat kidney cortex.

Dry Dowex 50 was left to swell for 24 h and then washed several times by decantation with water. The resin was transferred into 5×90 mm columns. Each column was purified by successive washing with: 80 ml $6N$ HCl, 15 ml H_2O, 80 ml $6N$ HCl, 15 ml H_2O, 40 ml $6N$ HCl and water to a neutral reaction of the eluate, the rate of flow due to gravity being 5 ml/h. This procedure took several days. The resin was then in the H^+ cycle and ready for use.

The test solution contained 7.3 μg Mg, 2.4 μg Ca, and further 5 μg Fe, 2 μg Zn, 0.5 μg Cu, 0.1 μg Mn and 0.523 mg KH_2PO_4 in 1 ml.

Elution of Mg and Ca. Three types of Dowex 50 with different cross-linkage were investigated for the separation of Mg and Ca. With increasing cross-linkage the strength of bond of Ca rose more rapidly than that of Mg so that, whereas both metals were eluted with $2N$ HCl in close sequence from Dowex 50-X4, Dowex 50-X8 permitted elution of Mg selectively with 5 ml $2N$ HCl, no Ca being present in the subsequent 4 ml of the eluate and appearing only after elution with 5 ml $3N$ HCl. On the other hand, the elution zones of Mg and Ca on Dowex 50-X12 were wide and diffuse.

Determination of Mg and Ca. After adding an aliquote of the test solution, the column of Dowex 50-X8 was washed with 5 ml $1N$ HCl; Mg was eluted with 5 ml $2N$ HCl and Ca with 5 ml $3N$ HCl. The eluates were collected in quartz tubes and evaporated in an electric oven at 100°C. The residues were dissolved in water.

Since the interfering metals are eluted together with Mg, they were removed by adding 0.1% 8-hydroxyquinoline (pH 5.6, acetate buffer) and extracting the chelates formed with chloroform[3]. Mg was then determined in the aqueous phase by titration with EDTA, using an Agla microsyringe, to Eriochrome Black T (pH 10.5, ammonium buffer)[4]. The equivalence point was determined graphically from curves of extinction changes followed on a Hilger Spekker photocolorimeter at 650 mμ.

Calcium was determined photometrically with sodium naphthalohydroxamate at 390 mμ (CF 4 Spectrophotometer) by the indirect method[2] or by complexometric titration to Calcon (pH 12.5, diethylamine)[5] similarly as

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