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Substance P, a Highly Active Naturally Occurring Polypeptide

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The study of naturally occurring biologically active substances is one of the most informative branches in medical research. These substances may be present or become active under normal and pathological conditions. In the first case they give us information on the mechanisms enabling the normal functions of the living organism; some of them are at the disposal of the physician as valuable drugs. In the latter case they may give us information about the origin of pathological processes. Research is further interested in these natural substances because knowledge of their chemistry, their metabolism and their mode of action enables one to look systematically for synthetic compounds which would act specifically on definite steps of normal or pathological processes.

The great progress in protein chemistry during the last few years has led to brilliant results in the field of biologically active peptides. After elucidating the complicated chemical structure of insulin and ACTH, intensive efforts are being made in synthetizing these substances. The two pituitary hormones, the cyclic octapeptides oxytocin and vasopressin, are at the physician's disposal as synthetic compounds. The naturally occurring vasoconstrictor angiotensin[1–3] was recognized as a linear octapeptide and synthetized; its physiological and possible pathogenic significance is still uncertain[4]. The same is true for the synthetic linear nonapeptide bradykinin[5] and the very similar decapeptide kallidin[6–8], both being strong vasodilators possibly playing an important part in inflammatory processes[9–12]. The first four peptides mentioned are hormones in the strict sense, whereas angiotensin, bradykinin and kallidin may be classified as tissue hormones and have in common the fact that they are released enzymatically from precursor substances in body fluids.

In the last few years Substance P (SP) another naturally occurring peptide has gained more and more interest. Recent progress in the field of this polypeptide makes it desirable to summarize our present knowledge.

1. *Initial studies on SP.* The first paper on SP was published in 1931. VON EULER and GADDUM[13] while studying the distribution of acetylcholine in tissues, made the observation already reported previously by other authors that acid alcoholic extracts of certain tissues of the horse contained another substance besides acetylcholine and histamine which also stimulated the isolated intestine of the rabbit and had a short lasting depressor effect in the atropinized rabbit. The substance has been found in large quantities in the intestine and the brain of the horse. The dried extract was simply named P (= powder) by the authors and so its active principle became known as SP. In the following years this substance was primarily investigated by two research groups, GADDUM in England and VON EULER in Sweden. They agreed on a standard preparation for comparative purposes, one unit of SP corresponding approximately to the amount of the active principle obtainable from 25–50 mg of horse intestine. On sensitive guinea-pig ileum preparations the average threshold dose is 0.05 to 0.2 U/ml. VON EULER[14] isolated SP from intestine and brain of cattle. He found that the substance could also be extracted by boiling the tissue in water at pH 4, that it was soluble in water, resistant to boiling at pH 1 to pH 7 but rapidly destroyed in alkaline conditions. He further observed that SP was inactivated by trypsin and therefore concluded that it was a polypeptide. He proved the presence of SP in biodialysates from rabbit intestine and presumed that this substance had a physiological significance for the motility of the intestine. GADDUM and SCHILD[15] reported that SP migrates to the cathode. GERNANDT[16] observed the stimulating effect of SP on

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the peristalsis *in vivo*. In 1953 PERNOW published his extensive studies on SP as a monograph[17]. He had already achieved a rather advanced purification. His procedure is in short the following:

Extraction by boiling the tissue for 10 min at pH 4, precipitation of inactive protein by adding ethanol and further concentrating the active substance by precipitation with ammonium sulfate. These crude extracts show an activity of 3 U/mg. They are then dried and dissolved in methanol and put on an aluminium oxide column on which the whole active material is adsorbed. The SP is eluted by methanol in diminishing concentrations whereby the peak of activity appears in the more diluted methanol or the water fraction. The dried eluate with the peak of activity contains an average of 100 U/mg. With a second chromatography on the same column but using ethanol PERNOW reached an activity of approximately 500 U/mg. The most active fraction for many years was obtained by distribution chromatography of the pretreated extracts on a cellulose column with butanol acetic acid as the solvent. The peak fraction had an activity of 300 U/mg and with Ninhydrin gave a slightly positive spot on the paper chromatogram.

The only difference between SP from intestine and brain was the higher lability of the substance obtained from the brain. Trypsin and even more so chymotrypsin inactivated this purified preparation. The purest products obtained by PERNOW had the smooth muscle stimulating as well as the blood pressure lowering, effects of crude extracts.

2. *Distribution of SP.* PERNOW[17,18] studied the content of SP in the *intestinal tract* of different mammals. The content is small in the oesophagus and stomach, larger in the duodenum and largest in the jejunum. It is less in the ileum and large again in colon and rectum. In the whole intestinal tract the muscularis mucosae contains the bulk of SP, whereas the layers of the intestinal wall devoid of ganglionic cells have almost none. On the whole there is a good correlation between the motility of an intestinal segment and its content of SP. The results of PERNOW are in agreement with those of DOUGLAS, FELDBERG, PATON, and SCHACHTER[19]. In Hirschsprung's disease EHRENPREIS and PERNOW[20] found practically no SP in the distal dilated parts of the rectosigmoid devoid of ganglionic cells, whereas the content was normal in the proximal segments with normal peristalsis and containing nerve cells.

In the *nervous system* it is striking to see that in all species studied so far, high activities of SP were found. It is present in human *brain*, in the brain of mammals, birds, reptiles, and fish[21–24] and is localized especially in the cell-rich phylogenetic oldest parts of the brain. The topographic distribution of SP in the central nervous system of humans and mammals has been studied by several authors and their results are more or less the same. PERNOW[17] found the highest values in the hypothalamus, thalamus and the basal nuclei of the dog and lower values in the cerebral cortex and the cerebellum. Large amounts are also present in the floor of the fourth ventricle of cattle and cats[25]. High SP values were reported by ZETLER and SCHLOSSER[26] to be present in the ala cinerea and in the substantia grisea centralis. According to these authors high SP values are associated with low cholinesterase values. This fact led to the hypothesis of SP being a noncholinergic transmitter. AMIN, CRAWFORD, and GADDUM[27] found high amounts of SP in most tissues rich in serotonin and norepinephrine. Especially high amounts were reported for the area postrema, probably a conglomeration of chemoreceptors, as well as for the nuclei of GOLL and BURDACH. According to DUNÉR, VON EULER, and PERNOW[28] the retina of cattle and dogs contains large amounts of SP.

In the *spinal cord* much higher SP values are found in the gray than in the white matter and more in the dorsal than in the ventral roots[17,29]. All peripheral nerves and peripheral ganglia contain SP[17].

The *subcellular distribution* of SP has also been studied. According to LEMBECK and HOLASEK[30] the greatest part of SP in homogenates of cat brain is found in the mitochondrial fraction. INOUYE and KATAOKA[31] investigated the subcellular distribution of SP in the brain and spinal cord of rabbits and guinea-pigs, and recovered the greatest amounts in the fraction of the 'synaptic vesicles' and in the supernatant (= microsomes and cytoplasmatic fraction) but small amounts in the mitochondrial and nuclear fraction. VON EULER and LISHAJKO[32] found SP in a bound form in subcellular granula of peripheral nerves.

GULLBRING[33] described the *enzymatic inactivation* of SP by protein containing extracts of horse intestine

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and basal ganglia of man and horse. EBER and LEMBECK[34] showed an enzymatic destruction of SP—probably by action of cathepsin—when incubating it with crude extracts of various organs. An SP inactivating fraction was extracted from kidneys of pigs[35]. KRIVOY[36] was able to inhibit the SP destroying activity of brain extracts by adding lysergic acid diethylamide (LSD).

3. *Changes of SP content in different organs due to different functional states or drugs.* PERNOW and WALLENSTEN[37] observed increased SP values in human intestinal segments when their motility was increased by intraluminal application of hypertonic glucose solution. VON EULER[38] reports that vagal stimulation increases the SP content of rabbit gut.

That SP might be involved in neuronal activity was demonstrated by HOLTON[39,40]. She showed in degeneration experiments on peripheral nerves that the SP content increased in the proximal part of the cut nerve while it decreased in the distal part. ZETLER and OHNESORGE[41] suggested that SP in the brain of mice occurs in two fractions behaving differently during the extraction procedure. They presumed that the SP content depends on the activity of the brain: it was decreased during anaesthesia and increased during central excitation. PAASONEN and VOGT[42] tried to change the SP content of the dog brain by drugs. Of the various sedatives investigated such as reserpine and of the centrally stimulating drugs none was able to change the SP content in the important parts of the brain. SERAFIMOV and STERN[43] were not able to find any alterations of SP content in rat brain after giving neostigmine, epinephrine, norepinephrine, serotonin, histamine, factor I and GABA, while i.v. application of SP increased the SP content of the brain. STERN and KOCIC-MITROVIC[44] could increase the SP content of the rat brain by reserpine; they believed that the sedative effect of reserpine could at least be partly due to its effect on the amount of SP in the brain. KOCIC-MITROVIC[45] reported a decreased SP content in pregnant rats which was explained by an increased SP destroying activity of the serum. STERN and KOCIC-MITROVIC[46] studied the effect of light on the SP content of the retina of cattle. In the covered eye, higher amounts of SP were found than in the uncovered eye. In the brain of rats and rabbits exposed to light or darkness, the changes of the SP content of the brain were opposite to those of the retina of cattle[47].

We have been interested ourselves[48] in the question of whether changes in the SP content of the brain could be induced by centrally active drugs. The brain extracts of rats given various sedatives and central stimulants were tested for their smooth muscle stimulating activity on the isolated ileum of the guinea-pig. Our experiments led us to the conclusion that none of the different extraction procedures used allowed an accurate determination of SP and that the elimination of other smooth muscle stimulating substances in the brain extracts[49] would require relatively complicated methods. Therefore, we are very careful in judging the SP values of brain given in the lit rature. In experiments similar to those reported by GADDUM[50] using a push-pull-cannula we looked for a possible release of SP in the nuclei of GOLL and BURDACH during electrical stimulation of the posterior columns in cats, but came to the same negative results.

4. *Pharmacological effects of crude SP extracts.* PERNOW et al.[51,52] have made extensive studies of the effect of SP on the *intestinal tract* and the circulation in man. The i.v. infusion of small amounts of SP causes strong peristalsis in healthy persons. In patients with paralytic ileus SP infusions were able to evoke considerable peristaltic waves. This effect, however, disappeared when the infusion ceased.

The *blood pressure* is briefly lowered; a peripheral vasodilation can be observed as a flush especially in the head. A strong tachyphylactic phenomenon limits the duration of the hypotensive effect so that the blood pressure becomes normal within a few minutes inspite of continuous infusion.

In humans the infusion of SP does not seem to have any effect on the *central nervous system* (PERNOW, personal communication). In animals, however, many central nervous effects of crude SP extracts have been described. VON EULER and PERNOW[53] observed an increased depth and rate of respiration together with a slight increase of blood pressure in anaesthetized rabbits and cats when given SP intraventricularly. Unanesthetized rabbits developed mydriasis and showed decreased spontaneous motility. The latter effect was also observed with most unanesthetized

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cats; similar effects were produced by giving 10 γ acetylcholine intraventricularly. Higher amounts of SP (100 U) led to a long lasting state of stupor.

The numerous publications on the central nervous effects of SP may be classified into two main groups of action, namely a 'general tranquilizing effect' and the postulated role of SP as a neurotransmitter in the sensory pathway.

A sedative effect i.e. a reduced spontaneous activity of animals has been reported several times[54–57]. SP has been said to have a taming effect on the Siamese fighting fish *Betta splendens*[58] and wild hares[59]. STERN and HUKOVIC[60] found that SP prolongs the sleeping time induced by hexa-barbitone. In this connection reports on anticonvulsive effects of SP may be mentioned: suppression of epileptic convulsions in mice caused by audiogenic stress[61], antagonistic action against central effects of harmine, strychnine, pikrotoxin, metamphetamine and morphine[56,62]. Comparing SP with bradykinin ZETLER[56] found the former active against harmine-induced tremor in mice, whereas it potentiated bulbocapnine-induced catatonia. In all these tests bradykinin had no effect. KISSEL and DOMINO[63] found that SP had no influence on mono- and polysynaptic reflexes. STERN et al.[64] on the other hand described an inhibitory effect of SP on polysynaptic but not on monosynaptic reflexes and they, therefore, believed in a synergistic action of SP and mephenesine in the cat. According to STERN[65] SP would not affect conditioned reflexes of the rat. BELESLIN, RADMANOVIC, and VARAGIC[66] reported that smaller doses facilitated while higher doses inhibited the ganglionic transmission in the superior cervical ganglion of the cat. CASPERS[67,68] studying the zero potential of the superficial cortex and the dendrite potential of the neocortex in rats, postulated that the effect of SP applied directly on the cortical surface is due to the hyperpolarisation of the generator structures in the superficial layers of the cortex. This hyperpolarisation could be due to a transmitter function of SP at inhibitory synapses or to a general and permanent stabilising effect of SP on neuronal membranes.

LEMBECK[29] considering the distribution of SP in the nervous system put forward the hypothesis of SP being the transmitter of sensory neurones. EEG-studies of cortex and hippocampus, showing an arousal reaction in rabbits following injection of SP in the carotid artery are in accord with this hypothesis[69]. LEMBECK[70] also described a stimulating effect of SP on the sensory nerve endings in the rabbit's ear. ZETLER[56] and PRZIC[71] found an antagonistic effect of SP on morphine-induced analgesia and respiratory depression. Some of the reported observations do not permit a classification of SP into one or the other of the above mentioned groups. ZETLER, therefore, assumed that SP acted as a neurotransmitter in sensory as well as in central inhibitory neurones.

All these studies were carried out with very impure preparations which according to our own experience contained considerably more of other substances than SP, whereby high salt concentrations, the presence of lipid soluble active tissue components and other biologically active peptides[72] may have led to false conclusions. Doubts about most reported SP effects are all the more justified since STERN and HUKOVIC[60] have found very crude and slightly purified SP preparations to have opposite effects in the same tests.

5. *Recent attempts to isolate SP.* The pioneering work of PERNOW[17] has already been mentioned. Recently FRANZ, BOISSONNAS, and STÜRMER[73] reported the isolation of SP from horse intestine:

The crude preparation obtained by ammonium sulfate precipitation according to PERNOW was freed of lipid soluble substances with ether and petrol ether and further purified on four different chromatographic systems (amberlite IR-45, amberlite IRC-50 (XE 64), aluminium oxide and DM-cellulose). The end product had an activity of 30000–35000 U/mg. Some amino acids are given in the paper. The physico-chemical behaviour of the product was that of a single substance and the authors therefore concluded to have isolated SP in pure form.

Our own work[74] in isolating SP started with material from cattle as well as from horse intestine.

We followed the method of PERNOW up to the chromatography on aluminium oxide. The fractions thus prepared had an activity of 100 U/mg when derived from cattle intestine and about 1000 U/mg when derived from horse intestine. For further purification a counter current distribution system containing

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n-butanol/pyridine/acetic acid/water (600:150:75:675) was used. After 1000 and more transfers a preparation containing 5000 to 10000 U/mg was obtained from cattle intestine, the yield being 70–90%. The molecular weight was found to be 1650 ± 350. After total hydrolysis the following 13 amino acids were found: lysine, arginine, aspartic acid, glutamic acid, proline, glycine, alanine, valine, leucine, isoleucine, phenylalanine, threonine, and serine.—Using the same distribution system a preparation of approximately 50000 U/mg was obtained from horse intestine. This could not be purified further by increasing the number of transfers. On paper chromatography the products from cattle and horse intestine were not distinguishable. After total hydrolysis the same amino acids were found in horse material. At that time this equine material was the most active SP preparation ever found. However, several observations made it clear that the substance could not yet be in a pure state. Tryptophane and some other partially biologically active material could be separated from SP by a second counter-current-distribution using the biphasic system *n*-butanol/acetic acid/water (4:1:5). The most active fraction (120000 U/mg) still shows a certain contamination with biologically inactive material as seen by the overlapping of the curves of biological activity with those obtained by colour reactions; thus the activity of absolutely pure SP will probably be somewhat higher than 120000 U/mg.

Comparing the properties of our purest SP preparations from horse intestine with those of the preparation believed by FRANZ, BOISSONNAS, and STÜRMER to be pure SP, one doubts the identity of these two products derived from the same material. The substance prepared by FRANZ et al. has a different migration velocity in the electrical field from our product, it does not, according to recent information of BOISSONNAS, contain threonine[75] and its activity appears too low for pure SP (this however might be due to differences in the standard used). The greatest difference in our opinion, however, seems to be the lack of lability of their substance in aqueous solution, a property characteristic of our highly purified SP.

Identity of our substance from horse intestine and of that prepared from cattle brain by ZUBER and JAQUES[76] is, however, more probable. The activities of the two preparations can only be roughly compared, because these authors did not use the international standard but only the variable threshold doses. The amino acid composition, however, is the same for both substances, a surprising fact when considering the different origins of material.

The chemical isolation procedure of ZUBER and JAQUES[76] consists in extracting cattle brain according to PERNOW[17] and precipitation of the extract with ammonium sulfate; the material thus obtained is further extracted with glacial acetic acid and the active substance precipitated with ether. Further purification requires repeated fractionations on Sephadex-G-25 and oxycellulose (elution with monochloroacetic acid) as well as on carboxymethyl Sephadex (C-25). The final step is a high-voltage-electrophoresis at pH 9,5.

6. *Assay methods for SP.* SP can only be estimated by biological methods; the stimulating effect on smooth muscle preparations as well as the depressor effect are used.

The problem of the biological assay of SP has been studied especially by GADDUM et al.[50,77]. The simplest and most sensitive test is done on the isolated terminal ileum of the guinea-pig. The isolated rabbit jejunum has the same order of sensitivity. The isolated rectal caecum of the hen is somewhat less sensitive[78] and the test is not so easily performed as with guinea-pig ileum. The rat's uterus is much less sensitive to SP. Moreover the main effect of impure SP preparations on the rat's uterus seems to be due to other components[50]. GADDUM[79] described a micromethod using the isolated goldfish intestine. In this preparation the SP effect may be overshadowed by other active substances present, as e.g. in the guinea-pig brain. In assaying impure preparations, care has to be taken to avoid effects of possibly present amounts of acetylcholine, histamine or serotonin, by adding atropine, a serotonin antagonist and an antihistaminic to the bath. The effect of possibly present bradykinin can be excluded by using the hen's rectal caecum which is either insensitive to, or relaxes in presence of bradykinin. The depressor effect on the blood pressure of the atropinized rabbit is a very sensitive test. The lack of a clear dose-response-relationship in the submaximal range, however, allows only the estimation of the threshold dose.

The comparison of a pure substance with a standard, in which the substance in question is present together with others, some of which are also biologically active, raises a basic pharmacological problem which will not be dealt with here. We would only like to mention that even highly purified SP preparations, e.g. with an activity of 50000 U/mg may contain other biologically active components. Such a component—in this case with an inhibitory effect on the gut—could be separated by the second counter-current-distribution. The chemical nature of this inhibitory substance has not been investigated further, but it is not unlikely to be one of the acid phosphatides known to be responsible for the effect of the so-called 'Darmstoff' of VOGT[80].

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The high lability in aqueous solution of our highly purified SP has not been cleared up and has made the biological assay very difficult to perform.

7. *Biological activity of highly purified SP.* According to the studies made by PERNOW[17], STÜRMER and FRANZ[81] one could expect that the highly purified SP would have the same qualitative effects on smooth muscles and on the circulatory system as impure preparations. The above mentioned contradictory and partially incomplete communications concerning the central effects of crude SP preparations did however not allow any conclusions as to the possible central effects of our pure SP. We therefore, carried out some preliminary investigations with SP of 50000 U/mg.

(a) The effect found on the *smooth muscle of the gut* confirms the previous findings of various authors. In sensitive ileum preparations 1 ng/ml bath fluid (1 ng = 10^{-9} g) produced a submaximal (approximately 70%) contraction. The dose-response-curves for the highly purified fraction and the impure standard (250 U/mg) show that our purification procedure led to the isolation of that component of the impure extract which is responsible for practically the whole activity of the extract on the guinea-pig ileum. In this organ SP is about ten times as active as acetylcholine and has about the same activity as bradykinin. The strong stimulating effect of SP on the gut has also been observed in the dog *in vivo*.

(b) *Circulatory effects.* In the rabbit the i.v. injection of 20 ng/kg of the highly purified fraction causes a short lasting drop of the blood pressure of about 20 mm Hg. In this test SP is about 100 times more active than acetylcholine. In the cat, somewhat higher doses of SP are necessary to obtain a similar hypotensive effect. Increasing the SP dose prolongs the duration of the depressor effect, whereas its intensity changes only little. As was shown by DUNÉR and PERNOW[51] for man, the tachyphylactic effect also appears in the animal. In the conscious dog the i.v. injection of a high dose of SP (7500 U/kg) leads to a drop in blood pressure lasting several minutes and to a remarkable bradycardia still present after the return of the blood pressure to normal. At least partially, SP acts by peripheral vasodilation which has been demonstrated in the isolated rabbit ear. A direct effect on the myocardium could be excluded for the isolated guinea-pig auricle and the papillary muscle of the rabbit.

(c) In the guinea-pig i.v. administered SP in a dose of 50 γ/kg had a clear *bronchoconstrictor* effect.

(d) Concerning the effect of highly purified SP on the *central nervous system* we have never been able to obtain an effect whatsoever with so remarkably few units as were formerly reported to be active with crude extracts. No effect was observed in mice with i.v. injection of up to 1000 U per animal. With 5000 U per animal however, the spontaneous activity was reduced in most mice. This effect lasted from a few minutes to one hour. When the animals were disturbed they reacted absolutely adequately. No influence on strychnine convulsions was observed with i.v. and s.c. injections of SP. In order to exclude a lack of central activity of SP due to restricted blood-brain-permeation of the polypeptide, we also injected it intracerebrally into mice (right parietal hemisphere). With up to 1000 U per animal no effects were observed; with higher doses the symptoms were the same as with corresponding doses given i.v. We could not find any influence of SP on the action of subthreshold doses of ethanol. In the unanesthetized dog the i.v. injection of the relatively high dose of 7500 U/kg (= 150 γ/kg) produced an immediate salivation lasting about 30 sec. During this period the dog always appeared frightened and restless which was probably due to the simultaneous drop of blood pressure. A few minutes later, the dog became drowsy, yawned, the eyelids seemed heavy and the dog obviously became indifferent to his environment. Some 15 min later his behaviour seemed quite normal again.

We also investigated the effect of our highly purified SP fraction on the ganglionic transmission in the superior cervical ganglion of the cat by retrograde injections into the lingual artery. Even very high doses proved to be ineffective. However, SP had a slight potentiating effect on epinephrine-induced contractions of the nictitating membrane.

No qualitative differences between the effects of our purest SP preparation from cattle intestine (10000 U/mg) and those of the highly purified equine material (50000 U/mg) were found.

8. *Differentiation of SP from other principles with similar biological actions.* SP can easily be distinguished from *kinins* (bradykinin, kallidin etc.)[82], although their effects are in part very similar. The presence of the so called *'Darmstoff'* of VOGT in the gut and its strong smooth muscle stimulating activity led to much confusion; 'Darmstoff' is now known to be a mixture of acid phosphatides[80,83] which lacks the depressor effect of SP. *Prostaglandin* extracted by VON EULER from prostate glands and seminal vesicles seems to be chemically related to 'Darmstoff'[84,85]. *Cholecystokinin* has not yet been characterized chemically; it differs from SP by a more specific effect on the gall bladder[86,87]. Neither has *Villikinin* been isolated in a pure form.

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This principle is believed by LUDANY[88] to be responsible for the motility of the intestinal villi. *Eledoisine*, isolated from salivary glands of octopodes by ERSPAMER[89] and synthetized by SANDRIN and BOISSONNAS[90] is an undecapeptide which has several actions in common with SP, especially the depressor effect on circulation and the strong stimulating effect on salivation.

9. *Conclusions.* SP is a basic polypeptide with a molecular weight of about 1600 containing 13 different amino acids. It is found in large amounts in the intestinal tract and in the nervous system of man and of all the vertebrates so far studied. Its distribution in the central nervous system shows close similarity with that of serotonin and the catecholamines which are probably playing an important part in the normal function of the central nervous system.

SP is the most active of the known naturally occurring substances stimulating intestinal smooth muscle and lowering the blood pressure. A physiological significance of SP for the intestinal motility seems probable. The topographic and subcellular distribution in the nervous system suggests a participation of SP in neuronal transmission processes. Its function as an actual transmittor substance as, e.g. in sensory neurones is, however, rather unlikely. The effects on central mechanisms of small doses of SP administered parenterally, as reported by various authors using crude extracts, could not be confirmed by us for highly purified material. It may well be that still unknown substances present in the crude extract are responsible for the contradictory effects reported. Actually, SP in much higher doses had a sedative effect in mice and dogs.

Recently three independant laboratories have succeeded in isolating SP in a rather pure form from horse intestine and bovine brain. Definitive conclusions concerning the physiological significance of SP will not be possible before synthetic SP is available for pharmacological purposes.

Zusammenfassung. Substanz P (SP) wurde als körpereigenes Polypeptid erstmals 1931 von v. EULER und GADDUM beschrieben und durch ihre glattmuskelstimulierende und hypotensive Wirkung charakterisiert. Sie kommt beim Menschen und bei den meisten untersuchten Tierspezies vor. Ihre Verteilung im Körper – Intestinaltrakt, peripheres und zentrales Nervensystem, vor allem dessen phylogenetisch ältere Teile – gleicht jener biologisch aktiver Amine und lässt somit an eine physiologische Bedeutung in der Regulierung der Darmmotilität und der Nervenaktivität denken. Verschiedene pharmakologische Wirkungen roher SP-Extrakte auf das Zentralnervensystem wurden beschrieben.

Kürzlich gelang drei Forschergruppen unabhängig voneinander die Isolierung von SP in ziemlich reiner Form aus Pferdedarm und Rinderhirn, und es ist wohl in absehbarer Zeit mit deren Aufklärung zu rechnen.

Es werden einige biologische Eigenschaften eines hochgereinigten SP-Präparates (50000 E/mg) aus Pferdedarm beschrieben. Das basische Polypeptid mit einem Molekulargewicht von ca. 1600 enthält 13 verschiedene Aminosäuren. Es ist die aktivste bisher bekannte Substanz mit stimulierender Wirkung auf die glatte Muskulatur. Ihre hypotensive Wirkung am Blutdruck des Kaninchens ist stärker als die von Acetylcholin. Bei intravenöser Verabreichung bewirkt SP beim Meerschweinchen eine Bronchokonstriktion. Was die Wirkung auf das Zentralnervensystem betrifft, waren zur Erzeugung eines leichten sedativen Effektes viel höhere Dosen des hochgereinigten Präparates nötig, als von verschiedenen Autoren für rohe Extrakte angegeben wurde. Die von verschiedenen Autoren postulierte Rolle von SP als Überträgerstoff im sensiblen System ist wenig wahrscheinlich. Ein eindeutiger Nachweis von Veränderungen des SP-Gehaltes im Gehirn durch zentral wirksame Pharmaka ist bisher nicht erbracht worden.

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The Influence of Diuretics on the Absorption of Salts, Glucose, and Water from the Isolated Small Intestine of the Rat[1]

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The mucosal cells of the small intestine actively pump sodium ions from the lumen into the submucosal space (HEIDENHAIN[2], INGRAHAM and VISSCHER[3], BURNS and VISSCHER[4], VISSCHER and INGRAHAM[5,6], CURRAN and SOLOMON[7], RUMMEL and STUPP[8]). Like

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