Symposium on substance P

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THE RELATION OF SUBSTANCES P TO NEUROTRANSMITTER SUBSTANCES

Some years ago I came to conceive the opinion, that an aqueous extract of dorsal roots contains a substance, which is a compound of a polypeptide with the transmitter substance of sensory nerves, and which has a high P-activity on the guinea pig ileum. With an enzyme, that can be obtained from nervous tissue, this compound can be split in an astonishingly short time into the polypeptide and the transmitter substance of sensory nerves. By this procedure the capillary dilatatory and reddening action on the denervated rabbit ear is not altered, but the P-activity on the guinea pig ileum is reduced to a low degree, which is about the same as that of an aqueous extract of ventral roots (Umrath, 1953, 1956). I will call this enzyme »Pease«. Since some sensory nerves, like the optic and the stato-acoustic nerve, have as low P-activities as the ventral roots, I tried to solve the question, whether their transmitter substance is different from that of the fibres in the dorsal roots.

In a series of rabbits one ear was denervated and the other left as a control. As it is known from previous work, the sensitivity of the denervated ear to extracts of dorsal roots is increased, but its sensitivity to ACh, H, ATP and to extracts of ventral roots is reduced (Hellauer and Umrath, 1948; Florey and McLennan, 1955). Now it turned out, that after denervation, at least in the first three weeks, the sensitivity for extracts of the optic nerve is decreased. So it is to be concluded, that the optic nerve possesses a transmitter substance different from that of the dorsal roots. I will call the transmitter substance of the optic nerve opticin and that of the dorsal roots dorsin. Only dorsin can be tested on the denervated rabbit ear, but both are effective in the bee-test of Florey (1951). This test uses positive phototactic bees, with cut wings, and with one eye punctured, in a faintly illuminated room. Such a bee takes an irregular course, without prefering the one or the other side. If a small quantity of an extract of dorsal roots or of optic nerve is applied to the punctured eye, the course of the bee deviates to the side of the treated eye, as if this eye were more illuminated than the other. Actually these substances, which enter the punctured eye, are the same as those normally produced in the eye by light.

Using these test methods it is possible to investigate the enzymatic breakdown of these transmitter substances. Working with aqueous extracts of the optic nerve, one finds what one would expect from analogy with ACh and acetylcholinesterase. Opticin is destroyed by an enzyme, opticinase, which is contained in the optic nerve in such a concentration, that by adding unboiled, crushed material from the optic nerve diluted 1:200, the effect of opticin in the bee-test is destroyed in about one hour. One gets nearly the same result with a 1:200 dilution of crushed nervous system from arthropodes or from annelides. If one takes dorsal roots (1:200) as the enzyme, opticin is destroyed in about 2 hours.

For dorsin I had three methods of preparing the solution. (1) Boiling 1 part of dorsal roots in 3 parts of salt solution. (2) Boiling, as before, 1 part of dorsal roots in 3 parts of salt solution and adding, after the decanted extract has cooled »Pease« (1 part of original nervous tissue to 1,000 parts of extract of dorsal roots); in about 10 minutes the action of »Pease« is completed and the extract is subsequently boiled for several minutes in order to destroy the »Pease«. (3) Boiling 1 part of dorsal roots with 3 parts of ethanol, decanting, evaporating the ethanol at 30°C and at reduced pressure, and dissolving the residue in 3 parts of salt solution. The P-activity on the guinea pig ileum of solution 1 is high, those of solutions 2 and 3 are low to an equal degree and nearly as low as the P-activity of extracts of ventral roots or of optic nerve. Only in solutions 2 and 3 dorsin is readily destroyed by dorsinase.

In the bee-test, using crushed dorsal roots (1:200) as the enzyme, the dorsin activity in solutions 2 and 3 is destroyed in 1—2 hours; using nervous system of arthropodes (1:200) the activity in solutions 2 and 3 is also destroyed in 1—2 hours, and, using crushed optic nerve, in 3—6 hours. In contrast to this, in solution 1, using the enzyme from dorsal roots (1:200), the dorsin activity in the bee-test is only destroyed after 4 hours.

In the test on denervated rabbit ear the apparent time intervals required for destruction are 2—3 times longer than in the bee-test. The reason may be that the residual solution after the action of dorsinase has an antagonistic action to dorsin in the bee-test, but a synergistic action to dorsin in the test on the denervated rabbit ear. If one would perceive, in the bee-test, the destruction of $^1/_3$ of the dorsin, and in the test on the denervated rabbit ear the destruction of $^2/_3$ of the dorsin, this would explain the differences in time. In using the test on the denervated rabbit ear one finds the dorsin activity in solution 2 and 3 destroyed by crushed dorsal roots or arthropode nervous system (1:200) in 3—5 hours, but in solution 1 by crushed dorsal roots only in 10—18 hours and by arthropode nervous system, in the same time interval, only in a barely detectable amount.

From these findings I conclude, that free dorsin exists in solutions 2 and 3, that can be destroyed equally well by dorsinase of the ver-

tebrate and of the arthropode nervous system, but that there is a dorsin-polypeptide compound in solution 1, with a high P-activity on the guinea pig ileum, which is not destroyed by dorsinase, so that the dorsin must be split off the polypeptide by »Pease«, before dorsinase can act. As it is known (Umrath, 1953, 1956), it takes much time to form »Pease« from crushed nervous system.

I imagine, that all the transmitter substances are bound to polypeptides, if stored in the nervous elements. The exception in the case of dorsin would be only, that its polypeptide-compound would not be split by boiling in water. In many cases the polypeptide compound is the precursor of the transmitter substance, in other cases it seems to play itself the role of the transmitter substance. According to von Oer (1961) one has to assume an ACh-compound, which is not destroyed by cholinesterase, as the transmitter substance from the epithelium of the cornea to the sensory nerves of the cornea and from secondary sense cells to their sensory nerves. Since we have seen, that the dorsin-polypeptide compound is not destroyed by dorsinase, it is very likely, that an ACh-polypeptide compound, not destroyed by cholinesterase, is the transmitter from the epithelium of the cornea and from secondary sense cells to sensory nerves.

Most work on SP was done with SP from intestine. It is available in crude extracts and in purified preparations. In both forms its P-activity is reduced by »Pease« in a short time to a lower level, which is not further decreased by longer exposure to »Pease«. From this I conclude, that SP from intestine is a polypeptide with a prosthetic group, which can be split off by »Pease«. SP from the intestine is active in the test on denervated rabbit ear, but this activity is not altered by »Pease« and almost not altered by trypsin. With an impure trypsin I could reduce the P-activity of a purified SP from intestine, estimated on the guinea pig ileum, to 2% of the original activity, reducing thereby the activity on the denervated rabbit ear to about 50%. From these findings I conclude, that the prosthetic group is mainly responsible for the activity on the denervated rabbit ear, but this activity is not destroyed by dorsinase, as it is in the case of SP from dorsal roots, the dorsin-polypeptide compound. A more obvious difference is, that SP from intestine has no activity in the bee-test, even at high concentrations of purified preparations, whereas, as already mentioned, SP from dorsal roots, the dorsin-polypeptide compound, has a good activity in the bee-test.

Summary

SP from dorsal roots is supposed to be a polypeptide with a prosthetic group, which is dorsin, the transmitter substance of the sensory nerve fibres in the dorsal roots.

SP from nervous elements not containing dorsin is supposed to be a polypeptide, which before extraction bore the transmitter substance of the neurons as a prosthetic group. Extraction with boiling

ethanol yields a polypeptide without a prosthetic group from all nervous elements, including dorsal root.

SP from intestine is supposed to be a polypeptide with a prosthetic group, different from dorsin, but similar to it in certain respects.

ODNOS SP I NERVNIH TRANSMITORA

Pretpostavlja se da je SP iz dorzalnih korjenova polipeptid s prostetičkom grupacijom. Ova grupacija je identična s dorzinom, transmitorom senzornih vlakana u dorzalnim korjenovima.

SP iz nervnih elemenata što ne sadrže dorzina smatra se polipeptidom, koji je prije ekstrakcije bio vezan s prostetičkom grupacijom identičnom s transmitorom odnosnih neurona. Pri ekstrakciji ključalim etanolom otcjepljuju se prostetičke grupacije svih nervnih elemenata, uključujući i one dorzalnih korjenova. Za SP iz crijeva se pretpostavlja da nosi prostetičku grupu različitu od dorzina, ali po nekim efektima sličnu ovom transmitoru.

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DISCUSSION

LISSAK: May I ask professor Umrath what is his opinion about the role of ACh content of sensory nerves (n. opticus, dorsal roots), whether he accepts the standpoint of O. Loewi and Hellauer, or mine and that of Brecht and Corsten? Have you tested the »Opticine« content of the retina? It is well known that the retina has an exceptionally high ACh content.

UMRATH: The figures for the ACh content of sensory nerves given by Loewi and Hellauer are about the same as those given by you or by Brecht and Corsten. Loewi thought, that such a small ACh content in sensory nerves is insignificant and so Loewi and Hellauer summarized, that sensory nerves contain no ACh. There can be no doubt, that in sensory nerves as a whole small quantities of ACh are present, but the question is, whether every sensory fibre contains a small amount of ACh, or if the total amount of ACh belongs to a small number of cholinergic fibres. An investigation on trophic influences of neurons in the nervous system by me and Hellauer (Deutsche Z. f. Nervenheilkunde, 1951, 165, 409—429) showed, that after sectioning the sciatic nerve, ACh in the central stump is augmented to 132%, calculated on wet weight of nerve, or, as the nerve stump gains on weight, to 161% calculated on even lenght of nerve. After removal of an eye from a rabbit we found the ACh content in the residual optic nerve and in the crossed optic tract to be augmented on the 4th day in 3 experiments to 158%, 161% and 226% calculated on wet weight of nerve. As the afferent, sensory fibres of the optic nerve have their cell-bodies in the retina and therefore degenerate after removal of the eye, our results, as I think, show conclusively, that the ACh in the optic nerve is located in a

small number of efferent, cholinergic fibres. In our experiments the sheath of the optic nerve was not removed and the ACh content of the normal control nerve was 0,5 μg/g.

To account for the ACh content of dorsal roots, there are, in my opinion, enough parasympathetic pathways leaving the spinal cord by the dorsal roots. Their cholinergic preganglionic fibres leave the spinal cord by the dorsal roots and, in the dorsal root ganglia, join the cholinergic ganglionic neurons, which send their axons through the dorsal roots and autonomous nerves to the innervated organs.

We have not yet tested the opticin content of the retina, but. as Dr. Vogt pointed out, the retina has several different neurons and therefore may have several different transmitter substances.

STÜRMER: We agree with Prof. Umrath's statement that SP is not always completely destroyed by incubation with trypsin. Our highly purified SP preparation retained some biological activity after incubation with trypsin for three hours at 25°C.