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THE RELATION BETWEEN »SUBSTANCE P« AND GLYCINE

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Substance P (SP) is a polypeptide that has been known since 1931 and consequently represents the first natural polypeptide which is commonly known. Gaddum and von Euler, who discovered it, drew attention, as far back as 1931, to the fact that SP has some similarities with acetylcholine and histamine, i. e. it contracts smooth muscle and increases the permeability of capillaries. During the period of 25 years there has been relatively little work done on the physiology and pharmacological action of SP. In the sense of an intensive investigation the whole thing was started only in 1953, in the work of Pernow[2] and Lembeck.[3] The first drew attention to the specific distribution of SP in the brain, and Lembeck to the interesting fact that SP is practically only found in the posterior roots of the medulla spinalis (MS). He then proposed the hypothesis that SP could have a certain role in the sensory transmission of stimuli, and in 1956 Zetler[4] showed that this substance probably has an inhibitory function in the transmission of stimuli from the posterior to the ventral horn of the MS. Zetler also especially drew attention to the many other central effects of SP. In short we can say that this polypeptide which is found in the intestine and brain of vertebrates has numerous central and periferal effects into which we cannot go here, but draw attention to the revies references, Lembeck and Zetler (1962),[5] Christensen and Haley (1966),[6] and our reference (1969).[7]

SP is obtained today in a pure form so that 1 mg contains over 100,000 units and chromatographically gives a single spot. The first to obtain the pure substance were Franz et al (1961),[8] followed quickly by Vogler et al. (1962)[9] and Zuber and Jaques (1962),[10] and finally by Baile and Meinardi (1967)).[11] The method of purification of SP which was suggested by Baile and Meinardi means a great advance, because it is more economic and the product which is obtained is more stable than that of the other above mentioned authors. The amino acids which are found in SP are all known but their sequence is not known, and neither has their synthesis been carried out.

We called SP a physiological tranquilliser in one reference,[12] having come to this conclusion on the basis of our experiments. We could show that SP sedates fighting cocks[13] and betta splendens fish[14], and also sedates wild rabbits,[15] fighting mice[16] etc. We could not confirm Zetler's findings that SP acts as an anti- strychnine agent. It appears that impure products which have less than 100 units/mg. really contain an anti-strychnine compound, while the pure product with more than 100 units/mg does not have this effect;[17] this was afterwards confirmed by Gaddum et al.[18] However we could confirm that the impure and pure fractions of SP act sedatively on animals and that it has an anti-analgetic action.[16]

The problem of the central action of this polypeptide is the subject of lively discussions in science. We,[7] together with Zetler,[4], Krivoy,[19] and some other authors, have produced several contributions which argue on behalf of a central effect of SP. However Haefely and Hürlimann[20] think that SP does not act so on the CNS if it is injected into animals.

They injected completely pure SP and certainly noticed the reduction of spontaneous motility in the mouse, but they do not think that is enough foundation to speak about the sedative i. e. central action of this polypeptide. We showed in one reference that in order to measure the sedative effect of one substance, looking from a pharmacological aspect, it is necessary primarily to experiment with excited animals[16] and as we have already mentioned SP really sedates fighting mice which were excited by isolation.[21] A mouse sedated in this way becomes fighting again if desmethylimipramine is applied to it, a known, quick-acting-depressant,[22] which surely argues for the action of SP on the CNS.

In this work we ware interested in the relation between SP and other transmittors in the MS. Today it is thought that apart from the biogenic amines, acetylcholine, histamine, serotonine in the CNS, some amino acids particularly glycine and GABA very probably have a transmitting function.[23] Both these amino acids cause an inhibitory function when they are iontoforetically introduced to the interneurones of the MS. Because Davidoff et al.[24] showed that the oclusion of the aort, which in the cat leads to spactic paralysis,[25] is followed by the loss of interneurones and leads primarily to the loss of glycine, we thought that it would be interesting to examine the relation between glycine and SP, and GABA and SP particularly in the CNS. It was necessary for the same reasons to see the relation between SP and these amino acids on isolated organs.

Erspamer and Anastasi[26] divided the polypeptides which act on smooth muscle into two main groups; so called Tachikinines which includes SP, Eledoisin, and Physalemin and Bradykinines which includes Bradykinine and Kallidine. Curtis et al.,[27] divided the amino acids which have a transmitting function into »glycine-like« (glycine, α-alanine, β-alanine, serine, taurine) which can be antagonised by strychnine, and »GABA-like«, which includes GABA, γ-amino-β-hydroxybutyric acid and E-aminocapronic acid, which are not antagonised by strychnine. We were interested in the action of these polypeptides in their relation towards amino acids with a transmitting function, centrally and peripherly. Centrally, in order to see if a relation exists in connection with some basis central effects of SP, and peripheraly to see if these amino acids interfere in any way with the above mentioned polypeptides.

METHOD

The effects were examined, on the smooth muscle of the ileum of the guinea-pig, of the following polypeptides in the presence of amino acids. Too fractions of SP (13 units/mg in the strength and 43 units/mg isolated from brain), 0,1 units/ml Physalemin (0,05 gama/ml), Eledoisin (0,01 gama/ml), Bradykinine (0,5 gama/ml), and Kallidin (o,5 gama/ml). From the »glycine-like« amino acids glycine, beta-alanine, alfa-alanine Taurine and serine were used, while the »GABA-like« amino acids, γ-amino-β-hydroxybutyric acid were examined. All the amino acids were given in the dosage 0,1 and 10 gama/ml. In the same way the effects were examined of acetylcholine and histamine in the presence of glycine. Acetylcholine was given in the dosage, 0,01 γ/ml, serotonine 0,1 γ/ml and histamine 0,01 γ/ml. All mentioned substances were examined on the ileum of male guinea pigs in a 20 ml bath containing Tyrode solution at 32°C.

We examined the central effects of the Tachi-kinines and Bradykinine groups of polypeptides on fighting mice and on the anti-analgetic effect. When male mice are isolated for 21 days in special cages, they become extremely agressive whether fighting or normal mice are put in the cages.[21] To such animals SP was given in a dosage 2000 units/kg i. p, bradykinine 10 γ/kg i. p. glycine[28], [23] (100 mg/kg body wt. i. p.). As GABA does not penetrate the haemoencephalytic barrier,[23], in contrast to glycine, we caused an increase in GABA in the CNS by giving amino-oxyacetic acid (20 mg/kg s. c.) a GABA-transferase inhibitor.[30] In all of these experiments, we also gawe strychnine (0,25 mg/kg s. c.) in order to see if it would cancel the effect of SP and glycine, in relation to bradykinine or GABA. We have already mentioned that the »glycine-like« amino acid effects can be antagonised with strychnine.[23]

The anti-analgetic effect was examined by giving mice morphine (5 mg/kg body wt. i. p.), and when, by means of the »hotplate«[31] we were certain that the analgetic effect had taken place, we gave SP or bradykinine in relation to glycine and amino-oxiacetic acid and we again measured the analgetic effect. The dosage of the mentioned substances were the same as in the experiment with fighting mice.

RESULTS

As can be seen from Table I, glycine potentiated the action of all the Tachi-kinines in dosages of 0,1 γ/ml and 10 γ/ml i. e. it caused considerably stronger contractions of the small intestine of guinea pig. »Glycine-like« amino acids, α-alanine and taurine also acted in the same way. Taurine potentiated both fractions of SP and Physalemin, but had no effect on the contraction caused by Eledoisine, α-alanine, and taurine potentiated the action of SP and other Tachi-kinines only in the concentration of 10 γ/ml. Futher we see that »GABA-like« amino acids: GABA, and γ amino-β-hydroxybutyric acid even in the dosages 10 γ/ml do not potentiate Tachi-kinines. Only γ-amino-β-hydroxybutyric acid in concentration 10 γ/ml slightly potentiated the contraction of Eledoisin. Futher more from »glycine-like« amino acids, β-alanine and serine do not increase the effects of Tachi-kinines even in the concentration 10 γ/ml. Of all the amino acids that were used none potentitated the action of Bradykinine or Kalidine.

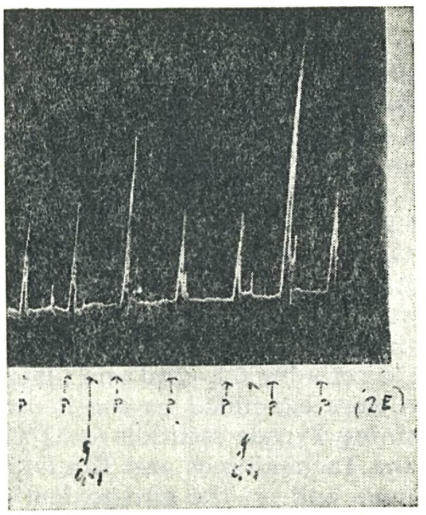

Fig. 1.

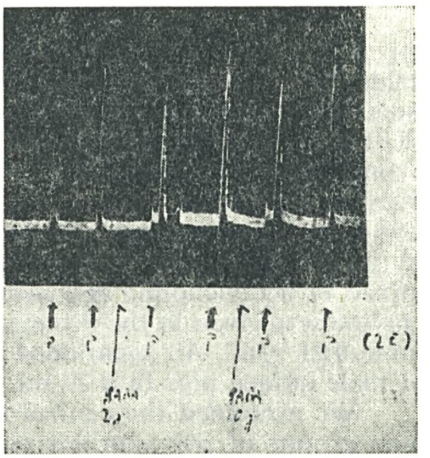

Fig. 2.

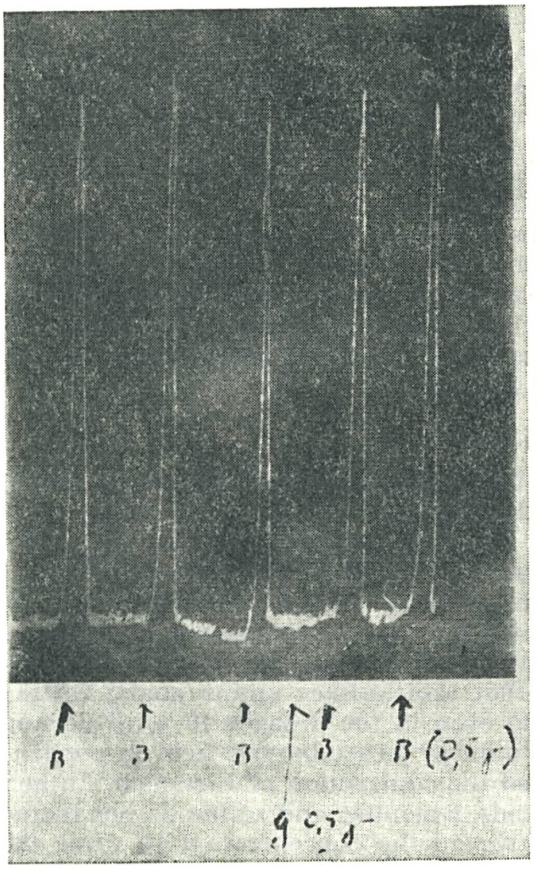

Fig. 3.

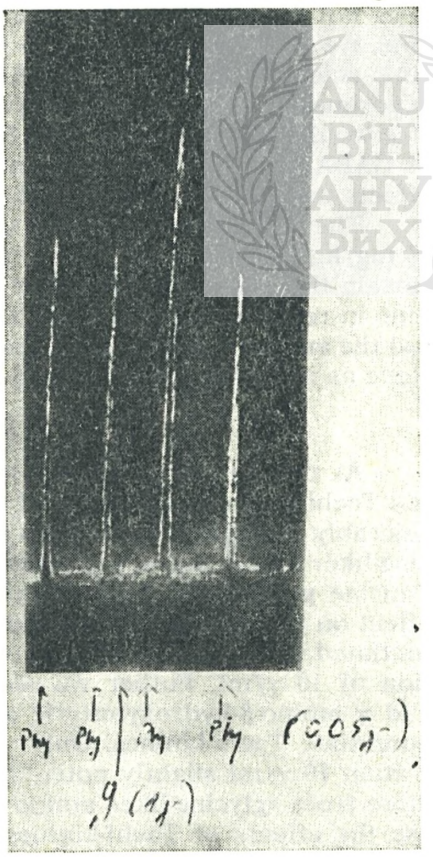

Fig. 4.

As glycine potentiated the action of SP, we examined the effects of this amino acid on other transmitting substances: histamine, serotonine and acetylcholine. It is shown that glycine even in the dosage of

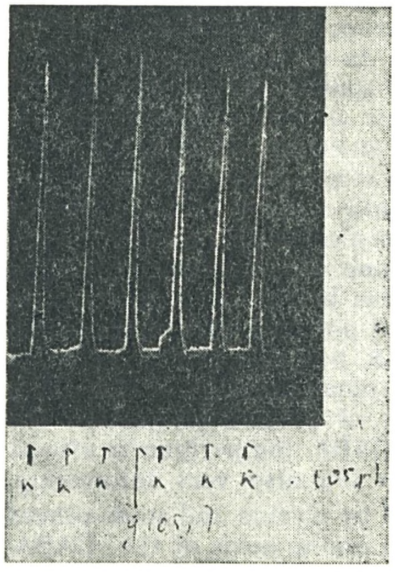

Fig. 5.

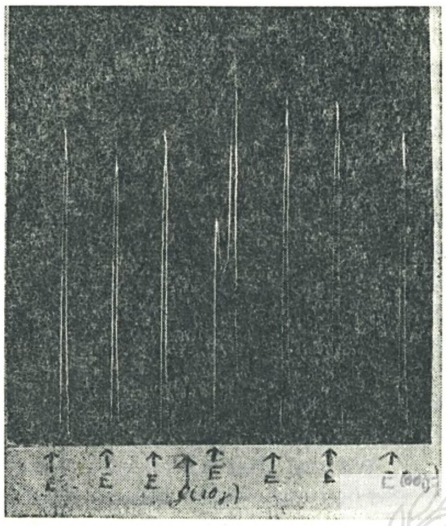

Fig. 6.

0,1 γ/ml potentiates the action of ACH (10^{-8}), while it does not potentiate serotonine (1^{-7}) or histamine (10^{-8}). If atropine is added (10^{-5}) preventively, the action of ACH is blocked, and the potentiating action of glycine on SP is unchanged.

PICTURES AND TABLES

As it can be seen from Table II SP and glycine sedate fighting mice, and they act anti-analgetically, while bradykinine and amino-oxyacetic acid are without effect. If strychnine is given to the mice at the moment when the fighting symptoms are not shown because of the application of SP or glycine, we can see that the fighting tendences reappear. Strychnine also annuls the anti-analgetic effect of SP and glycine. We see that bradykinine and amino-oxyaceticacid, which increased GABA in the CNS, have not even a sedative action on fighting mice or on the anti-analgetic effect.

DISCUSSION

As can be seen from these results SP and glycine act sinergisticaly. Both of these agents have a sedative action on fighting mice and both remove the analgesis caused by morphine. Bradykinine and GABA do not have this effect. We already amphasized at the beginning, that glycine disappears from the MS together with interneurones if ishaemia

of the MS which leads to spastic paralysis,[24] is caused for a certain time. The quantity of GABA in this experiment is not changed. Davidoff et al. supposed that the disappearance of the glycine is caused by the disappearance of the interneurones in the MS after ishaemia, while GABA either stays connected with some other neurones that do not disappear or it is not connected with neurones at all. As we have recently shown that glycine, in contrast to GABA, removed spastic paralysis caused by ishaemia of the MS in rats,[32], we think that we can suppose that glycine acts either by replacing SP if it is it's sinergist, or SP causing the liberation of glycine. SP alone is not able to remove this kind os spastic paralysis in rats. Several years ago we could show that SP does not disappear from the MS of rats below the cut-level.[33] This argues for the supposition that SP causes liberation of glycine. It is necessary to empasise that glycine is also widely spread in the brain,[34] and not only in the MS, so that this suppostion can be valid not only fort he anti-analgetic effect which unwinds throught the MS, but also for the sedative action, which is connected with the supraspinal function of the CNS.

In connection with the central effects of SP and glycine, or the lack of these, effects with bradykinine and GABA, the peripheral effects of the Tachi-kinines group of polypeptides are also very interesting.

We see that all three — are potentiated by glycine and some others of the »glycine-like« amino acid group. Both amino acids of the »GABA-like« group, GABA and γ-amino-hydroxy-butyric acid, are without effect. Bradykinine and Kallidine cannot be potentiated on isolated intestine of guinea-pig, but the amino acids of the »glycine-like« group do potentiate the action of acetylcholine on isolated intestine of guinea-pig. This effect can be easily removed by atropine, and the effect on polypeptides, i.e. potentiation by means of the »glycine-like« group, stays. It is well known that GABA exertet antiacetylcholine, antihistamine antiserotonin effects on the guinea-pig ileum.[35] According to this, this finding has it's value in relation to SP, not only for an explanation of it's central effects but because it in fact provides a new chanche of proving the specificity of SP. It is necessary to stress that Physalemine and Eledoisin are not found in mamals.[26] The potentiation of SP by LSD exists for instance,[36] but this psychopharmac also potentiats bradykinine.[37]

These experiments allow us to make the hypothesis that sensory impulses, either supraspinal or spinal, cause the liberation of SP which leads to the liberation of glycine, and glycine acts as an inhibitory neurone or any other neurone, for example in MS and — motor cell. In connection with that, SP is found in vesiculus of the nervous tissue of the dorsal part of the MS.[38] This also argues for the possibility that SP has a transmittor function. So we can explain why glycine removed the spastic paralysis caused by ishaemia of the MS. This hypothesis also agrees with our earlyer, findings that after the cut-level of MS of rat, the quantity of SP is not changed distantly.[33] In conection with effect of glycin on interneurons it is important to print out that glycin has no effect on Renshaw cells.[39]

P. ŠTERN I JELENA JELIČIĆ

ODNOS IZMEĐU »SUPSTANCE P« I GLICINA

KRATKI SADRŽAJ

Pokazano je da »supstanca P« i glicin uklanjaju osjet bola i agresivnost kod miševa, dok su GABA i bradikinin u tom testu bez efekta. Glicin i još neke amino-kiseline iz tzv. grupe »glycine-like« amino-kiselina koje imaju transmitornu funkciju potenciraju djelovanje »Supstance P« physalemia i eledoizina na izoliranom crijevu zamorca. Na istom testu GABA i bradikinin su bez efekta, t.j. ne potenciraju ni »Supstancu P« ni physalemin ni eledoizin. Na temelju ovih pokusa kao i nekih drugih autora zaključuje se da bi »Supstanca P« mogla djelovati tako da dovodi do oslobađanja glicina.

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EFFECT OF CERTAIN POLYPEPTIDES IN THE PRESENCE OF AMINO ACIDS ON THE CONTRACTION OF GUINEA PIG ILEUM

POLYPEPTIDE	CONCENTRATION	Amino acids							
		Glycine 0,1 γ/ml	Glycine 10 γ/ml	α-alanin 10 γ/ml	β-alanin 10 γ/ml	serine 10 γ/ml	taurine 10 γ/ml	GABA 10 γ/ml	γ-amino-β-hidroxy butyric acid 10γ/ml
Subst. »P« 13 U/mg	0,1 U/ml	+	+	+	—	—	+	—	—
Subst. »P« 43 U/mg	0,4 U/ml	+	+	+	—	—	+	—	—
Physalaemin	0,05 γ/ml	+	+	+	—	—	+	—	—
Eledoisin	0,01 γ/ml	+	+	+	—	—	—	—	±
Bradykinin	0,5 γ/ml	—	—	—	—	—	—	—	—
Kallidin	0,5 γ/ml	—	—	—	—	—	—	—	—

+ = increasing of concentration
— = without effect

	Fighting nouse	Analgetic's effect	Notice
Substance P	+	+*	* Strichnine excludes the effect
Bradykinin	—	—	
Glycin	+**	+*	** Effect apears 18—24 hours later
Amino-oxyacetic acid	—***	—	*** No effect even after 24 or 48 hours

+ = abolishing of fighting or analgesic
— = without effect