Symposium on substance P

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SPECIFIC ANTAGONISTS OF SUBSTANCE P

Many authors interested in the SP-field have tried to find its specific antagonists. Pernow (1953) in his monography discussed antagonists of SP particulary those belonging to the class of ganglion blocking agents but none of these was specific. The existence of specific antagonists of SP has very often been inferred in the literature, but as far as we know none has really been found, nor has any enzyme specifically destroying SP yet been isolated. Enzymes which have been found to destroy SP also destroyed other polypeptides and biogenic amines. No suitable specific pharmacological test for the identification of SP has been found either, except those using animal gut, particularly the guinea pig ileum.

Krivoy (1957), in Gaddum's laboratory, found that LSD potentiates the effect of SP and Elliasson (1958) in von Euler's laboratory succeeded to show that patuline inhibited the effect of biogenic amines leaving the effect of SP unimpared. These findings could be reproduced in our laboratory and partly enable us to identify SP.

We have been screening possible antagonists of SP for more than 6 years. A great deal of drugs have been included, which seemed promising on a theoretical basis. With respect to possible interactions with SP drugs could be divided in to 3 groups. (1) Drugs which are not at all, or are only unspecific, antagonists. (2) Drugs which are specific antagonists facultatively, and (3) drugs which are really specific antagonists. Having examined a great number of substances along these lines we wish to present the results of this work.

Methods

A piece of guinea pig ileum was suspended in an isolated-organ bath in Tyrode's solution. The temperature of the solution was 32°C. Air was bubbled through the bath and the substance to be tested was added. After recording the response, another piece of gut was used for the next antagonist.

The activities of antagonists against different agonists were compared on ground of Schild's pA_2 (1947). The pA_2 is defined as the negative logarithm, to base 10, of the molar concentration of an antagonistic

drug which reduces the effect of a double dose of an active drug (agonist) to that of a single dose.

The agonist was injected every 3 min., left 30 sec. and than washed out. The contractions were recorded with a frontal lever. 3—4 contractions were allowed, first with the single dose, and than one contraction with the double dose, 2 min. before applying the double dose the antagonist was added in such a concentration that a slightly higher contraction was obtained with the double, than with single dose. The piece of ileum was than changed for a new one and, after a period of adaptation of 15 min., 3—4 control contractions were recorded again, and the antagonist was given in such a concentration, that the double dose of agonist gave a contraction of slightly less height than the control single dose contraction. The height of the double dose contraction expressed as per cent of the last single dose contraction was plotted as the ordinate against the log. of molar concentration of antagonist. The 100% value was found by interpolation and the pA_2 calculated.

Solutions were made from different stock of SP, which had varying activities: 5.3, 16.3 and 270 U./ml, and (an SP prepared in our laboratory) 3—5 U./ml. Final concentrations of the active drugs for most of our experiments were: SP 0.50 U./ml (SP 5.3 U./ml kindly supplied by N. V. Organon, Holland), ACh, 0.02 μMol/ml, 5-HT, 0.10 μMol/ml, H, 0.04 μMol/ml, $BaCl_2$, 150μMol/ml, bradykinin 0.02 μg/ml.

Results

The series of drugs shown in Table I did not exert any inhibitory effect neither on SP, nor on other polypeptides and biogenic amines, in reasonable concentrations of about 1 mg/ml. There have been, in this series, drugs with unspecific inhibitory effects on the activity of SP and still more potent effects on biogenic amines, like atropine, scopolamine, quinine, tolserol and others.

TABLE I

ANTAGONISTS OF SP
UNSUCCESSFUL AND UNSPECIFIC ANTAGONISTS OF SUBSTANCE P

Adenosine, Adermine, gama-Aminobutyric Acid, 1-AMP, 2-AMP, Aneurine, Antimit, Antipernicin, Adenosine Triphosphate, Avacan, Azulen Benzochquinone, Biotine, B O L, Buscopan, Catechol, Citric Acid, Codeine, Coffeine, Cysteine, Decholin Sodium, Desoxyribonucleinic Acid, Eutison, Dilatol, Gluthathion, Heparine, Histamine, Hydrochloric Acid, Hydrasines, Lactoflavin, L S D, Marsilid (Iproniazid), Mephenesine, Meprobamate, 1, 2-Naphthoquinone, 1, 4-Naphthoquinone, alfa-Naphthylamine, beta-Naphthylamine, N-(Naphthyl)ethylendiamine, Narceine, Nepresol, Oxytocine (Syntocine), P A B A, Patulin, Phenuron, Poliethylen Sulfate, Protamin, Quiloflex, Quinine, 8-Hydroxyquinoline, Renin, Scopolamine, Segotin, Semicarbazide, Sparteine, Strychnine, Thiosemicarbazide, Trimethadione, Urea, Vasopressin, W-181.

Another, smaller series of drugs, were facultative inhibitors like 5-adenylic acid, narcotine and acetyl aneurine which were, from time

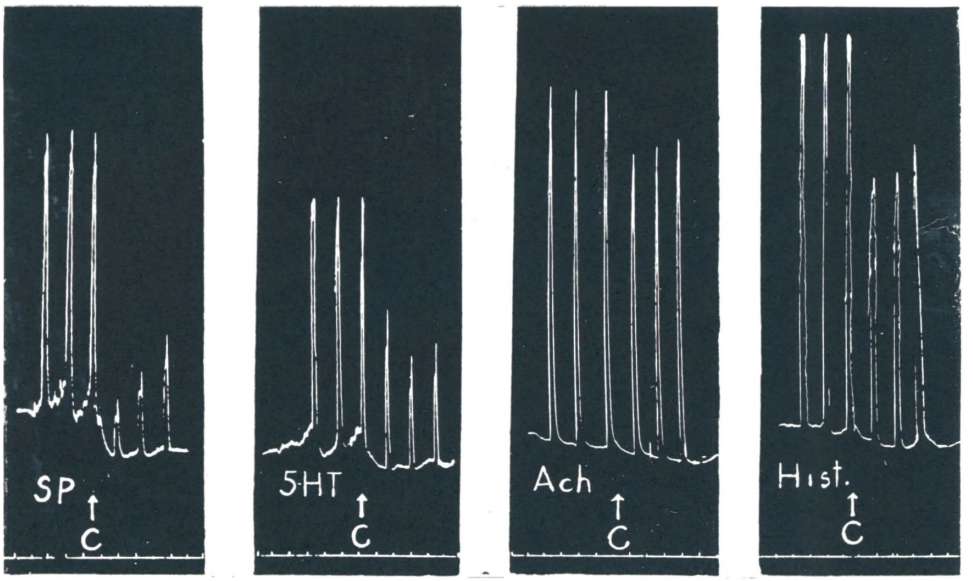

FIG. 1.

The effect of cysteine-di-β-naphtylamide (CdN) on the contractions of the isolated guinea pig ileum. Contractions are produced by active substances: SP, 0.5 U./ml; 5-HT, 10^{-6} Mol.; ACh, 2×10^{-8} Mol.; H. 6×10^{-8} Mol. The active substances are added every 3 min., left for 30 sec., then washed out. At the arrow, CdN (1.2×10^{-3}) was added and left for 2 min. The effect of active substance is then recorded, Contractions come back to the initial level slowly, after washing out.

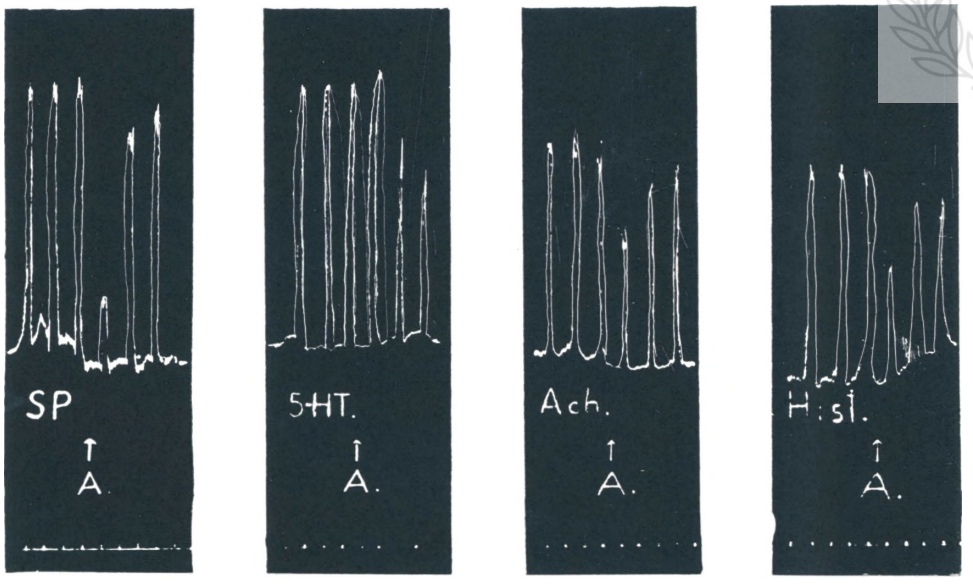

FIG. 2

The effect of Arfonad (A) on the contractions of isolated guinea pig ileum. Contractions are produced by active substances: SP, 0.5 U./ml; 5-HT, 10^{-6} Mol; ACh, 2×10^{-8} Mol; H, 6×10^{-8} Mol. The active substances are added every 3 min; left for 30 sec., then washed out. At the arrow Arfonad is added, and left for 2 min. The effect of active substance is then recorded. Contractions come back more quickly to the initial level after washing out than when CdN is used.

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to time and from animal to animal, more or less specific. pA_2 for this series is shown in Table II.

TABLE II

ANTAGONISTS OF SUBSTANCE P
pA_2 OF FACULTATIVE SPECIFIC ANTAGONISTS OF SP

Drug	SP	5-HT	ACh	H	Bradykinin	$BaCl_2$
5-Adenylic acid	5 3599	4.7510	3.7748	4.4150	4.8750	4.3400
Narcotine	4.8056	4.9420	4.1088	4.6910	—	—
Acetylaneurine	—	—	—	—	—	—

The third and most important series of drugs is that of the specific antagonists, which antagonized 4—5 times more SP than other polypeptides and biogenic amines. These drugs are: cysteine-di-β-naphtylamide and Arfonad. Hexamethonium could also be included into this group. The pA_2 for this series can be seen in the Table III.

TABLE III

ANTAGONISTS OF SUBSTANCE P
pA_2 FOR SPECIFIC ANTAGONISTS OF SUBSTANCE P

Drug	SP	5-HT	ACh	H	Bradykinin	$BaCl_2$
Cystin-di-beta--Naphthylamide	5.2499	4.7688	4.6036	4.5680	4.8170	4.0400
Arfonad	5.0300	3.9495	4.3685	4.5280	—	4.5550
Hexamethonium	3.5650	3.0000	3.0000	3.0000	—	—
2-Acet-Naphthalide	5.7450	5.5380	4.1700	4.2650	—	—

For reasons of qualitative comparison and control of the calculated values for specific antagonism the antagonistic effect of cysteine--di-β-naphtylamide is shown in Fig. 1. The effect of SP is the most inhibited of all other antagonists.

Arfonad inhibited the most SP in comparison with other active substances, like 5-HT, ACh and H as it has been shown in Fig. 2. It is interesting to note that the effect of 5-HT was potentiated after Arfonad. All active substances, after washing out of the antagonist reassume the former height of contraction.

Discussion

There are 3 groups of antagonists of SP. The first group includes unspecific antagonists, the second antagonists which are more or less specific, from animal to animal, and the third group is constituted by substances which are always the most potent inhibitors of SP as compared to other active drugs, which cause contraction of the guinea pig

ileum. The most active drugs in this series are cysteine-di-β-naphtylamide and Arfonad. The antagonistic effect of hexamethonium is not so easy to demonstrate, because great concentrations of hexamethonium alone cause the contractions of fresh ileum.

Tuppy (1959) showed that the substrate of oxytocinase is not only oxytocine but also cysteine-di-β-naphtylamide (CdN), a simple dipeptide. This relation of CdN to oxytocinase was in view of its peptide structure the reason for this investigation. It could be shown that this simple peptide inhibits SP more strongly than other active substances used in our experiment. The oligopeptide gluthathione did not possess this property. The moieties, also, of the CdN molecule, cysteine and naphtylamine are no specific inhibitors.

Pernow (1953) investigated the effect of ganglion blocking agents, like nicotine, tetraethylammonium and hexamethonium on SP activity. We have picked out Arfonad, which excedes the tetraethylammonium potency 30 times as the ganglion blocking agent to be assayed against SP. From our experiments it could be concluded that Arfonad has the same effect on SP like CdN and that Arfonad is also a specific antagonist. This substance is structuraly similar to biotine, but biotine did not show any antagonistic activity. Hexamethonium in a concentration of 0.40 to 1.0 M causes contraction of the guinea pig ileum by itself. On these contractions the contractions produced by SP did not superpose, but they do superpose on the contractions produced by biogenic amines. The stimulating effect of hexamethonium did not allow a straightforward conclusion of its exerting specific antagonism.

Laszlo (1960) proved that crude SP is accompanied, among other impurities, by 5-adenylic acid. This compound depresses the contractions of guinea pig ileum. It can be seen from our results that 5-adenylic acid reduces the effect of SP and active substances. The specificity varied from animal to animal and from time to time. We think that these variable results are caused by a different activity of adenosine monophosphatase, which destroys 5-adenylic acid. The difference probably lies in the animals, for we did not always have the same breed. The solution of 5-adenylic acid has to be made fresh, and used within a short time. Zetler (1959) noticed in his experiments that certain solutions of SP lost their protective activity, if they were left for more than 15 min. in solution.

The inhibitory potency of narcotine was the same with 5-HT and SP. This alcaloid could be used in a mixture of different antagonists, which do not inhibit 5-HT, but inhibits SP and some other biogenic amines. In this way a combination of 2 or 3 antagonists could be composed and an antagonistic effect against SP could thereby be achieved.

The most specific SP-antagonists from all drugs investigated in this experimental series are dissimilar in structure. The degree of inhibition and specifity is not so distinctive as the antagonism between atropine and ACh or LSD and 5-HT, but we think it will give us a clue to identify SP under certain conditions. These findings, also,

prompt us to try to find more specific antagonists among peptides and ganglion blocking agents.

Summary

Using Schild's pA_2 scale a series of substances were investigated as potential antagonists of SP. Three groups of substances could be combined. The first group is constituted of substances unable to produce any depression in reasonable concentrations. The drugs which are unspecific antagonists of SP are listed in the same group.

The second group comprises the drugs, which are more or less specific, from animal to animal. To this group belong: 5-adenylic acid, narcotine and acetylaneurine.

The third and the most important group are the specific antagonists. The most thoroughly investigated ones are cysteine-di-β-naphtylamine and Arfonad. These structuraly different substances have a quantitatively similar effect.

Specific antagonism is not so strong as that usually seen with biogenic amines and their specific antagonists, but it gives us some possibility to use the respective compounds as tools in further investigation, which should be turned towards peptides and very potent ganglion blocking compounds.

SPECIFIČKI ANTAGONISTI SUPSTANCIJE P

Istraživan je niz supstancija kao potencijalnih antagonista SP. Za ova istraživanja je upotrijebljena Schildova pA_2 metoda. Nađene su tri grupe supstancija. Prva grupa su supstancije koje ne izazivaju nikakvu inhibiciju u prilično velikim koncentracijama. U istu grupu su ubrojani nespecifični inhibitori aktiviteta SP. Druga grupa su supstancije, koje su više ili manje specifične na raznim životinjama i u raznim vremenskim periodima. U ovu grupu spadaju: 5-adenilna kiselina, narkotin i acetil-aneurin.

Treća i najvažnija grupa su specifični antagonisti. Najviše su ispitivani cistein-di-β-naftilamid i arfonad. Ove dvije strukturalno različite supstancije izazivaju kvalitativno slične efekte.

Specifični antagonizam prema SP nije tako uočljiv kao kod ispitivanja biogenih amina i njihovih specifičnih antagonista, ali nam naši rezultati daju mogućnost da upotrijebimo pomenute nove antagoniste SP kao sredstva za dalja istraživanja. Traženje novih antagonista SP treba da se orijentira prema peptidima i vrlo jakim blokatorima ganglija.

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DISCUSSION

GADDUM: I congratulate the authors on these results. Have you tried Tubocurarine?

HUKOVIĆ: We are going to try. Dr. Varagić tried Tubocurarine and found that it inhibits SP.

STERN: It would be very important to put new synthetic peptides (oligo and poly), as well as ganglioplegic agents to test as potential antagonist of SP. Possibly much stronger and more specific antagonisms will be detected in this manner.

LEMBECK: I have tried Dibenamine and found that it is not an antagonist of SP.