with 2-hydroxyethyl hydrazine to give 4-acetyl-1-(2-hydroxyethyl)-5-methyl-pyrazole, m.p. 138°; calculated for $C_8H_{12}N_2O_2$. C, 57.13; H, 7.19; N, 16.66%. Found: C, 57.32; H, 7.32; N, 16.42%; UV: λ_{max}^{ETOH} 246 nm (log ε 4.05); IR (nujol): 1658 cm^{-1}, 3290 cm^{-1}. Mannich condensation of the above pyrazole with 4-(*p*-fluorophenyl)-1, 2, 3, 6-tetrahydro pyridine hydrochloride and paraformaldehyde in the presence of catalytic amounts of hydrochloric acid affords the hydrochloride from which the base is liberated. The base (I), m.p. 148°, calculated for $C_{20}H_{24}FN_3O_2$; C, 67.20; H, 6.77; N, 11.76. Found: C, 67.38; H, 6.93; N, 11.64; UV: λ_{max}^{ETOH} 248 nm (log ε 4.39); IR (nujol): 1670 cm^{-1}. On treatment with citric acid in methanolic solution, the base forms the citrate, m.p. 136°, calculated for $C_{26}H_{32}FN_3O_9$; C, 56.82; H, 5.87; N, 7.65. Found: C, 56.86; H, 6.07; N, 7.47. UV: λ_{max}^{ETOH} 248 nm (log ε 4.41).

Zusammenfassung. 3-[4-(*p*-Fluorphenyl)-1, 2, 3, 6-te-trahydro-1-pyridyl]-1-[1-(2-hydroxyaethyl)-5-methyl-4-pyrazolyl]-1-propanon (I, CIBA 4416/B-Go) senkt bei normotonischen und hypertonischen Tieren den Blutdruck. Die Drucksenkung kann hauptsächlich auf die periphere Vasodilatation sowie auf die Vasomotorenzentren bezogen werden. Ausserdem wirkt CIBA 4416/B-Go adrenolytisch.

V. P. Arya, R. S. Grewal,
C. L. Kaul and S. P. Ghate

CIBA Research Centre, Goregaon East, Bombay 63 (India), 1 April 1971.

Antagonism of Dibutyryl-Guo-3′:5′-P and Atropine on Stomach Smooth Muscle Contraction

The effects of cyclic-adenosine-3′5′-monophosphate (Ado-3′:5′-P) and its dibutyryl derivative (db-Ado-3′:5′-P) on smooth muscle activity are well known[1-3]. Furthermore, basal and stimulated motility of the stomach in the dog, as well as in man, is reduced or abolished during db-Ado-3′:5′-P infusion[4], while blood perfusion pressure is unaffected. Smooth muscle relaxation can be associated with increased concentrations of Ado-3′:5′-P, induced by catecholamines through activation of β-receptors[5]. The α-receptor effects of catecholamines, however, could be mediated by a decrease of the intracellular Ado-3′:5′-P levels which in turn determines smooth muscle contraction[6]. Ball et al.[7] were able to demonstrate that α-adrenergic agents increase, in man, the extracellular cyclic-guanosine-3′5′-monophosphate (Guo-3′:5′-P). Also, it has recently been shown that, at least under some conditions, Guo-3′:5′-P activates the phosphodiesterase enzyme system responsible for the inactivation of Ado-3′:5′-P[8].

Cyclic-3′5′-guanosine-monophosphate is present in considerable amounts in various mammalian tissues, particularly in the gastrointestinal tract[9] and is affected by hormones and other factors, in a different way to Ado-3′:5′-P[10].

These findings and the results obtained by Manganiello et al.[11] in adipose tissue, where Ado-3′:5′-P and Guo-3′:5′-P act in opposite directions, prompted us to investigate the effect of the compounds on the gastric smooth muscles. The experiments were carried out on the rat stomach fundus strip prepared according to Vane[12].

Our results demonstrate that, also in this tissue, the 2 cyclic nucleotides act in opposite directions. While db-Ado-3′:5′-P antagonizes the effect of acetylcholine

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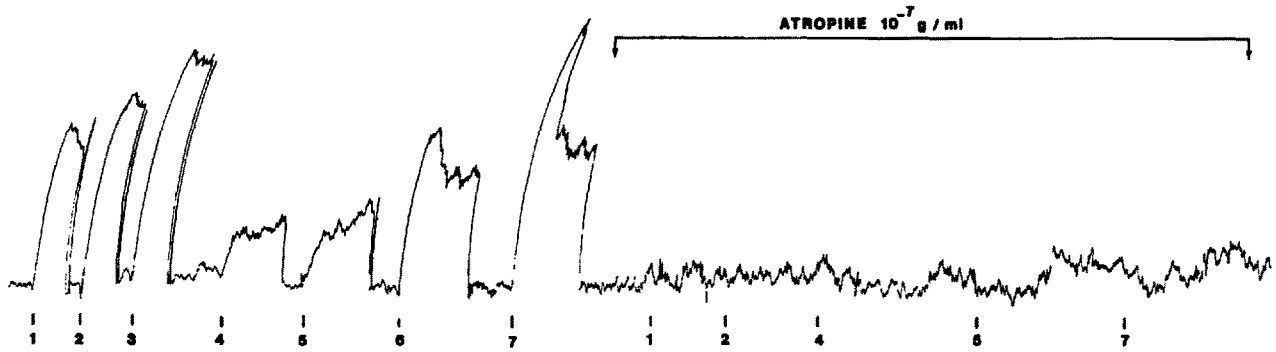

Effect of atropine sulfate on the action of acetylcholine-HCl (Ach) and dibutyryl-cyclic-3′,5′-guanosine monophosphate (db-Guo-3′:5′-P) on the isolated rat stomach fundus strip. 1, Ach, 4 µg/ml; 2, Ach, 6 µg/ml; 3, Ach, 8 µg/ml; 4, db-Guo-3′:5′-P, 125 µg/ml; 5, db-Guo-3′:5′-P, 250 µg/ml; 6, db-Guo-3′:5′-P, 375 µg/ml; 7, db-Guo-3′:5′-P, 500 µg/ml. Perfusion with Tyrode solution; bath volume 10 ml; temperature 37 °C. Atropine sulfate 1×10^{-7} g/ml was added to the tyrode solution for 60 min, after which, in its presence responses 1, 2, 4, 5 and 7 were repeated.

in a non-competitive way (unpublished data), db-Guo-3′:5′-P contracts the tissue in a dose-response relationship. Atropine blocks completely the contraction evoked by db-Guo-3′5′-P, as shown in the graph.

Separate experiments on stomach strips, obtained from rats previously vagotomized, as described by PATON and VANE[13] show that db-Guo-3′:5′-P has completely lost its contracting action. These results suggest that Guo-3′:5′-P is related to the mechanism of cholinergic transmission. In agreement with this hypothesis are the results of GEORGE et al.[14] on the acetylcholine (Ach) effect on isolated perfused rat heart: Ach increases Guo-3′:5′-P levels in this tissue and decreases those of Ado-3′:5′-P. MURAD et al.[15] have demonstrated that in homogenized dog hearts choline esters are inhibitors of Ado-3′:5′-P formation. Furthermore, FERRENDELLI et al.[16] have shown that oxotremorine treatment in mouse increases the Guo-3′:5′-P levels in cerebral cortex and cerebellum, an effect readily blocked by treatment with atropine.

On the basis of our results and these observations, it may be concluded that Guo-3′:5′-P is physiologically related to the autonomic nervous system. In order to clarify the mechanism of action of this naturally occurring nucleotide (Guo-3′:5′-P) and its relationship to the second sympathetic messenger (Ado-3′:5′-P) experiments on other smooth muscle activities are in progress.

Riassunto. Viene mettona in evidenza l'effetto contratturante del Guo-3′:5′-P sulla muscolatura liscia di stomaco di ratto in vitro. Tale effetto è antagonizzato dall'Atropina e scompare dopo vagotomia. Una possibile interazione fra il Guo-3′:5′-P e la trasmissione colinergica sembra così emergere da questi primi risultati.

L. PUGLISI, F. BERTI and R. PAOLETTI

Institute of Pharmacology and Pharmacognosy, University of Milano, Via A. del Sarto 21, I-20129 Milano (Italy), 8 March 1971.

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Antiviral Substances in Starfish

Starfish have been known to contain toxic substances in their tissues[1]. In 1960, HASHIMOTO and YASUMOTO[2] first reported the occurrence of saponin-like compounds in a Japanese species, *Asterina pectinifera*. Similar findings were reported with other Pacific and Atlantic species[3]. The saponins were described as hemolytic and cytotoxic[3a, b, 4]. Their actions to the nerve system were also discussed in comparison to a sea-cucumber saponin, holothurin A[5].

In the crude extract of a common Atlantic starfish, *Asterias forbesi*, the author found the presence of substances which are active in the antiviral test using influenza virus in chicken embryo. Further investigation confirmed the activity in many other species. Purification of the active components has been carried out with 3 species: *Acanthaster planci* (a South Pacific species), *Asterias forbesi* (a common Atlantic species) and *Asterias pectinifera* (a Japanese species). A standard procedure for the purification of the active components was established using the activity as guidance.

Ground air-dried starfish were extracted with chloroform. The chloroform extract was discarded. The residue was exhaustively extracted with a mixture of methanol and chloroform (1:1). The concentrated extract was dissolved in water and centrifuged. The supernatant was dialyzed to distilled water. The non-dialyzable fraction was lyophilized and chromatographed on a silica gel column. This operation afforded the active fraction as a mixture of several substances having very close Rf values on thin-layer chromatograms (TLC). The major components were further separated by repetition of dry-column chromatography and preparative TLC. The fractions which show single spots on TLC were passed through cation exchange resin (Na^+ form) and recrystallized from EtOH and Acetone-H_2O. The recovery of the substances from the chromatography was very poor, and only two each of the major components could be brought to purified form. The yields of the purified products were generally a few mg each from 100 g of the dried starfish. The isolated substances and their activities are listed in the Table.

The compounds are of highly glycosidic nature, and seem to coincide with the so-called asterosaponins reported by YASUMOTO and HASHIMOTO[2]. A relatively high yield of ASP-I from *Asterina pectinifera* enabled the author to obtain more information about the chemical nature of the compound.

Antiviral activities of purified fractions from starfish

Origin	Compounds	Melting points	Concentration (mg/ml)	Results
Asterias forbesi	AF-I	amorph	0.4	512
	AF-II	205–210°	0.5	16
	AF-III	218–223°	0.4	16
Acanthaster planci	AP-I	228–235°	0.4	16
	AP-II	215–219°	0.5	16
Asterina pectinifera	ASP-I	181–185°	0.3	128
	ASP-II	229–232°	0.3	16
				Control 4096

[a] Chick embryo technic was used. The inhibition of influenza virus multiplication by 1 ml of the test solution in an embryonated egg is expressed by the maximum dilution ratio of the allantoic fluid which causes hemaglutanation. The value 16 indicates the highest activity measurable by this assay.

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