Table III. Effect of amantadine on the concentration (μg/g ± S.D.) of dopamine, homovanillic acid and noradrenaline in rat brain

Group	Dopamine	Homovanillic acid	Noradrenaline
Control	0.18 ± 0.13 (4)[a]	0.03 ± 0.02 (9)	0.26 ± 0.08 (6)
Amantadine (25 mg/kg i.p., daily for 9 days)	0.20 ± 0.09 (4)	0.03 ± 0.01 (13)	0.23 ± 0.06 (12)

[a] Number of rat brains.

within the brain[11,12]. On the other hand, large amounts of amantadine inhibits the uptake of dopamine and noradrenaline by rat brain homogenates[13] and that of dopamine by blood platelets[13]; this, however, seemed to be rather unspecific and obviously without any physiological significance. The results of the present investigation did not demonstrate any significant changes in the concentrations of HVA and 5-HIAA in the CSF, which might reflect the metabolism of dopamine and 5-hydroxytryptamine in the brain[15]. The unchanged content of amines analyzed in the brain of a parkinsonian patient on amantadine therapy further supported this point. Correspondingly, amantadine did not alter the endogenous concentration of dopamine, noradrenaline or HVA in the brain of the rat. However, it must be taken into consideration that amantadine might have certain effects on the turnover of these amines in the brain without altering their endogenous concentration. Further studies will be necessary to elucidate whether a suggested amphetamine-like mechanism on monoamines[12] might account for the clinical efficacy of this drug against Parkinson's disease.

Zusammenfassung. Nachweis des stark herabgesetzten Säuregehalts der Homovanillinsäure und der 5-Hydroxyindolessigsäure im Liquor cerebrospinalis von Parkinsonkranken im Vergleich mit normalen Versuchspersonen. Die Amantadin-Therapie hingegen ergab keine bedeutenden Veränderungen und der Gehalt von Dopamin, Noradrenalin und Homovanillinsäure im Gehirn der behandelten Parkinsonpatienten entsprach auch den Werten der übrigen Parkinsonkranken. Ausserdem hatte Amantadin keinen Einfluss auf den endogenen Gehalt dieser Amine im Rattengehirn.

U.K. Rinne, V. Sonninen and M. Hyyppä

Department of Neurology, University of Turku, SF-20520 Turku 52 (Finland), 22 Oktober 1971.

Paradoxical Inhibition of the Effects of Bradykinin by some Sulfhydryl Reagents

Phenylbutazone and similar antiinflammatory drugs inhibit bradykinin induced bronchoconstriction in the guinea-pig[1], and the second phase of the hypotension evoked in rabbits and guinea-pigs[2,3]. The same drugs interfere with the action of SRS-A[4], arachidonic acid[5], and SRS-C[6,7], in guinea-pigs, rabbits and dogs. These and other results led to the suggestion that all agonists whose in vivo pharmacological effects are inhibited by analgesic antiinflammatory drugs released a common mediator, in a process where those antagonists interfere. A new principle, called 'rabbit aorta contracting substance' (RCS), released from guinea-pig lungs by bradykinin, SRS-A and anaphylaxis, has been proposed as such a mediator, as its release is blocked by aspirin and indomethacin[8,9]. We have recently shown that injection of SRS-C and arachidonic acid is followed by a release of RCS from isolated guinea-pig lungs and that this release is similarly blocked by aspirin and phenylbutazone[10].

The present results show that certain sulfhydryl agents, known to potentiate the vascular effects of bradykinin[11,12] and the non-sulfhydryl reagents pyrogallol and hydroquinone, also inhibit the in vivo bronchoconstriction due to bradykinin in the guinea-pig. Moreover some of those antagonists at convenient doses curtail the effects of bradykinin in the rabbit, as does phenylbutazone. Finally those drugs suppress the release of RCS from isolated guinea-pig lungs injected with bradykinin. New evidence is thus provided for the common mediator hypothesis and it is suggested that in the system responsible for the release of RCS an oxidative step is involved, which is blocked by antioxidants and by acidic antiinflammatory drugs.

Materials and methods. The blood pressure of rabbits and guinea-pigs anaesthetized with sodium pentobarbitone was recorded; guinea-pig pulmonary resistance to inflation was also measured by appropriate transducers on a pen recorder. Guinea-pigs were pretreated with propranolol to prevent antagonism of bronchoconstriction due to release of adrenaline[13]. All injections were given intravenously.

Release of RCS from isolated guinea-pig lungs was investigated as described by Piper and Vane[9] with simultaneous record of pulmonary resistance[10]. Isolated strips of rabbit aorta and pulmonary artery and of rat

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Drugs	Dose (mg/kg)	% Inhibition of bronchoconstriction (guinea-pig) [a]	Dose (mg/kg)	% Inhibition of hypotension (rabbits) [b]
DEDTC	25	50.8 (3)	25	65.1 (4)
	50	65.5 (3)	50	44.7 (4)
	100	81.6 (4)	100	15.9 (9)
			200	+ (3)
ME	50	52.3 (9)	35	+ (5)
	100	77.9 (17)	100	20.5 (6)
			200	90.0 (3)
TG	50	29.7 (2)		
	100	64 (25)	100	74.5 (5)
	200	79 (11)	200	74 (15)
Dithiothreitol	25	61.23 (1)	50	+ (3)
	50	37.3 (3)		
Pyrogallol	1	87.5 (3)	2.5	55.7 (5)
	2.5	100 (2)	5	79.4 (7)
			10	77.1 (7)
Hydroquinone	1	70.5 (3)	2.5	16 (6)
	2.5	100 (2)	5	70.5 (3)
			10	80 (4)

[a] Inhibition by sulfhydryl and antioxidant derivatives of bronchoconstriction due to bradykinin on the guinea-pig. DEDTC, sodium diethyldithiocarbamate; ME, 2-mercaptoethanol; TG, thioglycerol. Percent inhibition for groups of 2–25 animals was measured by comparing the area of bronchoconstriction before and immediately after the antagonist, for sulfhydryl reagents. In the case of hydroquinone and pyrogallol the antagonists were mixed with bradykinin at room temperature 15 min before and then injected. [b] Measured by comparing the area of hypotension before and after antagonists. Potentiation of responses to bradykinin at certain doses of putative antagonists is indicated by + in place of figure of % inhibition. Number of experiments between parentheses. All injections performed intravenously.

stomach were superfused with Krebs solution flowing from the lungs (10 ml/min) which were ventilated with $0_2 + CO_2$ (95 and 5%).

The following drugs were used: 2-mercaptoethanol (ME) and thioglycerol (TG) (Fluka); sodium diethyldithio carbamate (DEDTC) and dithiothreitol (Sigma); hydroquinone (Laboratories du Bois de Boulogne); pyrogallol (Koch-Light) bradykinin and eleidosin (Sandoz) and angiotensin II (Hypertensine, Ciba).

Results. The Table summarizes the results, showing that bronchoconstriction due to bradykinin (1–5 μg/kg) was inhibited by the sulfhydryl and antioxidant reagents (Figure 1 shows no effect on bronchoconstriction due to eledoisin[14] (0.5–2 μg/kg, 4 experiments); neither was the effect of serotonin (5–10 μg/kg, 5 experiments) inhibited, thus eliminating unspecific bronchodilator effects.

The hypertensive responses that follow i.v. injections of bradykinin to pentobarbitone anaesthetized guinea-pigs, were consistently inverted to hypotension. For example the area of hypertension, measured by planimetry, was of 4.71 ± 1.02 cm² in a group of 8 guinea-pigs, whereas after 100 mg/kg of ME a hypotension of 1.65 ± 0.3 cm² was apparent. Under treatment with the antagonists, once a hypertensive response had been converted to hypotensive, no return to hypertension was observed even after various injections of bradykinin whereas, depending on the dose of the antagonist, inhibition of pulmonary responses faded rapidly. A new dose of an antagonist blocked again the bronchoconstriction, but the hypotensive response to bradykinin was still more prolonged.

Bradykinin potentiating peptides (BPP5 and BPP9), which are synthetic peptides of 5 and 9 aminoacids, respectively, known to potentiate the effects of bradykinin[15,16], increased the bronchial constriction induced

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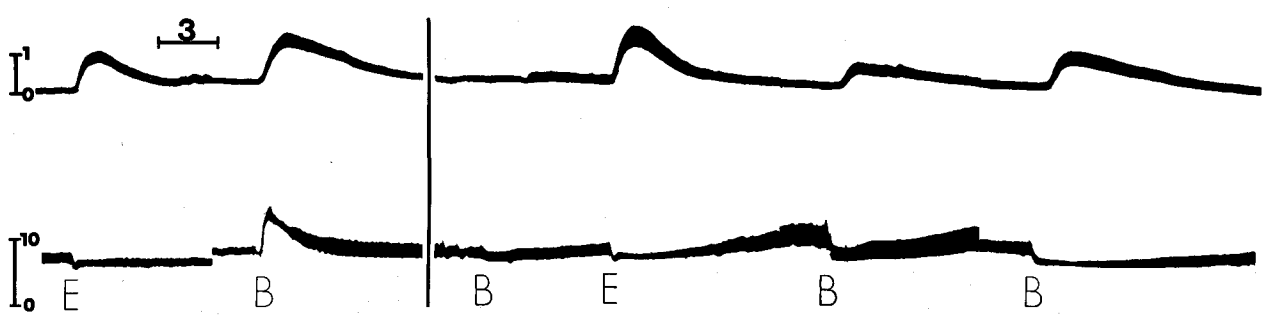

Fig. 1. Inhibition by 2-mercaptoethanol of bronchoconstriction due to bradykinin and ineffectiveness against eledoisin. Upper: pulmonary resistance to inflation (cm Hg). Lower: blood pressure (cm Hg). Horizontal bar: time (3 minutes). Male guinea-pig, 400 g. B, 1 μg/kg of bradykinin; E, 0.5 μg/kg of eledoisin. First panel (before vertical bar), and second panel (after vertical bar) respectively before and after 100 mg/kg of 2-mercaptoethanol. First injection of bradykinin was made 5 min after inhibitor. Interval between injections of agonists were of 15 min, between which recording was stopped. Observe inhibition of bronchial response to bradykinin, whereas eledoisin is unaffected.

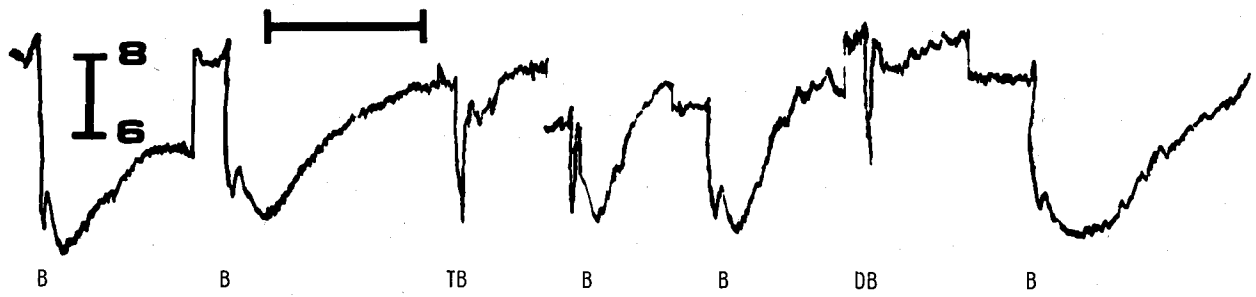

Fig. 2. Suppression by thioglycerol and by diethyldithiocarbamate of the secondary hypotension due to bradykinin in the rabbit. Injection of 0.5 μg/kg of i.v. bradykinin to a 2 kg female rabbit at B. At T and D 100 mg/kg of respectively thioglycerol and diethyldithiocarbamate. Observe curtailment of hypotension due to bradykinin after antagonists. Intervals between injections: 15 min; horizontal scale: 5 min; vertical scale: blood pressure in cm Hg.

by bradykinin by a mean of 6.51-fold for 2 mg/kg of BPP5 (5 experiments) and by a mean of 7.34-fold for 250 μg/kg of BPP9 (5 experiments). Despite this potentiation, the reference doses of the various antagonists inhibited the pulmonary responses.

Scarcity of BPPs precluded their use on a large number of control experiments. Nevertheless, that inhibition of pulmonary responses to bradykinin is effective, and not simply due to fading of the actions of BPPs, was indicated by the fact that hypotensive responses were prolonged, whether pulmonary responses were present or not at a given point in an experiment, again dissociating vascular and pulmonary responses to bradykinin.

Sulfhydryl reagents administered at doses of 50–200 mg/kg, and pyrogallol and hydroquinone at 5–10 mg/kg, curtailed the hypotension induced by 0.5–2 μg/kg of bradykinin administered to rabbits 1 min after the antagonist (Table and Figure 2). This shortening lasted for more than 1 h for the higher dose of TG, whereas in the case of ME and DEDTC, fading of the antagonistic activity was frequently followed by potentiation, which was again liable to shortening by any of the tested agents. This effect was probably due to the known potentiating effect of those metal chelators upon primary hypotension due to bradykinin[11,12].

Bradykinin injection into the pulmonary artery (5–10 μg) of the isolated lungs was regularly followed by the contraction of the isolated organs. It has been shown that these contractions are due to the release of RCS and of prostaglandins (whereas the direct effect of bradykinin is minimized by its pulmonary destruction[17]). Antagonists were initially superfused onto the isolated organs; thioglycerol and ME (20 μg/ml), dithiothreitol (40 μg/ml)

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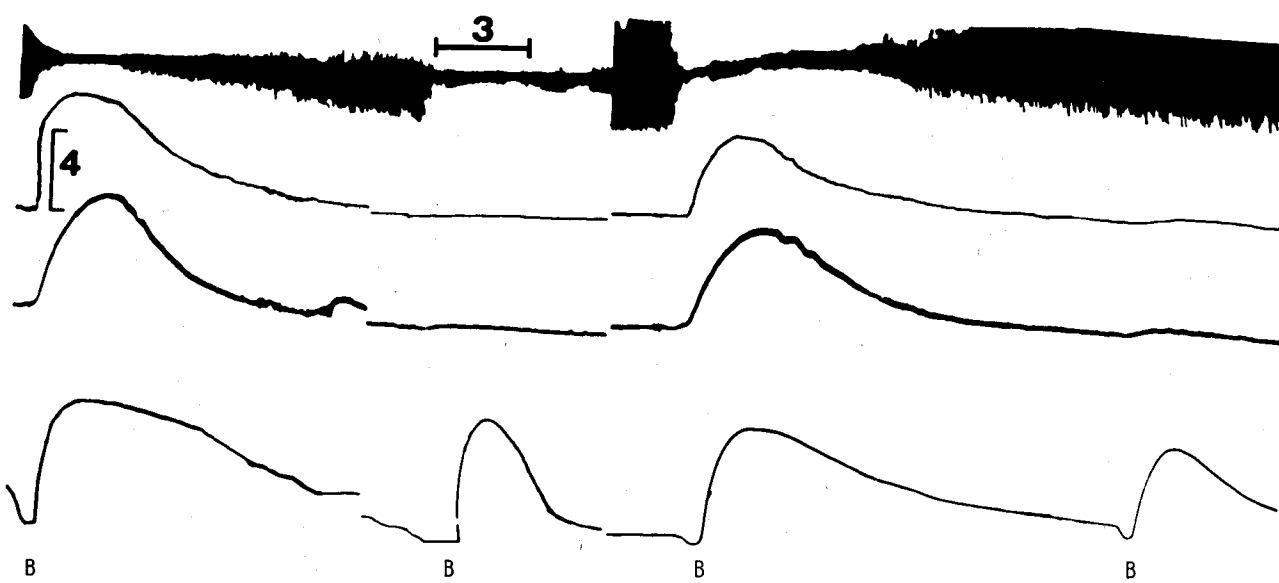

Fig. 3. Suppression by 2-mercaptoethanol of the release of RCS by bradykinin. Perfusion of the lung of a 200 g male guinea-pig. Upper to lower; record of the bronchial resistance, and of the responses of superfused rabbit pulmonary artery and aorta and rat stomach. Horizontal scale: 3 min. Vertical scale: 4 cm. At B 10 μg of bradykinin were injected. The 1st and 3rd injections were into the lung, and resulted in the outflow of vasoactive material, i.e., RCS. Organs were bathed with 40 μg/ml of 2-mercaptoethanol, that did not perfuse the lungs. The second injection was performared after 10 min perfusion of the lung with 40 μg/ml of mercaptoethanol. Finally the 4th injection was onto the isolated organs, demonstrating the direct effect of bradykinin on the rat stomach strip and absence of effect on the arteries. Observe total inhibition of release of RCS after lung perfusion with 2-mercaptoethanol, and residual contractions of rat stomach, which is larger than that due to direct effect of bradykinin. Bronchoconstriction is not affected by 2-mercaptoethanol.

and pyrogallol (5 μg/ml) did not affect the contractions of the isolated organs induced by angiotensin II (1–5 μg) or prevent their responses after the injection of bradykinin into the lungs, but completely blocked the release of RCS by bradykinin, when injected into the lungs 5–10 min beforehand (Figure 3). DEDTC (80 μg/ml) did not prevent the release of RCS.

Discussion. We have recently demonstrated[18] that sulfhydryl and antioxidant agents suppress the release of RCS, the hypotension and bronchoconstriction due to SRS-C and arachidonic acid. Blockade was obtained whether the antagonists were administered to the animals before SRS-C and arachidonic acid, or incubated for 15 min with the antagonists and then injected together.

The following hypothesis may be considered for the mechanism of action of the reference antagonists:

1. Rupture of vital S-S bridges belonging to an enzymatic system activated during the release of RCS: the relatively low activity of the otherwise strong S-S reducer dithiothreitol[19] and the effectiveness of hydroquinone and of pyrogallol justify caution in accepting such a straightforward explanation;

2. Interference of sulfhydryl and other antioxidants upon the receptor, as described for TG inhibition of effects of oxytocin[20] or for 2–3 dimercaptopropanol inhibition of rat gut contraction due to bradykinin[21].

3. RCS could be an unstable prostaglandin precursor[22], which is rapidly inactivated[9,10], whereas SRS-C (a mixture of unsaturated fatty acids[23]), arachidonic acid, and its peroxide[24] would be RCS precursor.

This hypothesis is compatible with the release of prostaglandins during experimental inflammation[26], with the blockade by non-narcotic antiinflammatory agents of the in vitro synthesis of prostaglandins from arachidonic acid[27] and with the suppression of the in vitro activity of SRS-C by triphenylphosphine, a lipid peroxide destructor[24].

Nevertheless, although they suppress in vivo bronchoconstriction and in vitro release of RCS, ME and the other antagonists did not prevent in vitro bronchoconstriction due to bradykinin (Figure 3). This apparent contradiction may be due to the direct spasmogenic effect of bradykinin, more important in vitro than in vivo, where the indirect mechanism predominates; alternatively, RCS may finally not be the common mediator of bronchoconstriction, but still be involved in the mechanism of action of antiinflammatory drugs.

No definite choice between those or other explanations is possible at this stage. It remains that the effects of bradykinin, of RCS-C and of arachidonic acid that are inhibited by antiinflammatory drugs are also suppressed by various antioxidants, reinforcing the hypothesis of a common mechanism and/or a final metabolic pathway for those agonists.

Résumé. Plusieurs dérivés thiol et deux antioxidants inhibent la bronchoconstriction chez le cobaye, une partie de l'hypotension chez le lapin et la libération de «rabbit aorta contracting substance» (RCS) dues à la bradykinine. Il est proposé que les effets communs à la bradykinine, à la SRS-A, SRS-C et à l'acide arachidonique, qui sont bloqués par des antiinflammatoires acides, et par les dérivés thiol, sont dus à la formation de RCS, constituée par des péroxydes cicliques analogues aux précurseurs instables des prostaglandines E 2 et F 2 alpha.

B.B. VARGAFTIG[28] and NGUYEN DAO HAI

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F-60 Eragny-sur-Epte (France),
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Cytoplasmic Uniaxial Radial Symmetry During the Early Final Growth Period in the Oocytes of the Japanese Quail (*Coturnix coturnix japonica*)

In previous work[1] we have established the existence of peculiar subcortical cytoplasmic organelles in the oocytes of regularly laying Japanese quails just before and/or at the beginning of yellow yolk formation. These RNA-rich organelles, although Feulgen negative[2], seem to contain freshly synthesized DNA which can be demonstrated by autoradiography after H-Thymidine injection[3]. In the present work their shape and distribution in the germinal disc has been studied.

Materials and methods. 1. *In toto study of germinal discs.* Laying Japanese quails were killed by decapitation, their abdomen opened and the intrafollicular peduncular oocytes, with a diameter ranging from 5 to 7 mm, removed from their ovaries by cutting off their pedicle. The freshly excised oocytes were placed in Ringer's solution and opened by a circular incision round the germinal disc. The yellow yolk and as much as possible the yolk underlying the germinal disc were removed. Thereafter, in a second bath of Ringer's solution, the theca interna and externa were peeled off from the membrana granulosa (follicle cell layer) overlying the germinal disc. The germinal disc supported by the membrana granulosa was then fixed in acetic acid-alcohol (1:3) for 1 h.

2. *Serial sections of quail oocytes.* After killing the birds by decapitation, the abdomen was opened and oocytes with a diameter ranging from 4.5 to 8 mm were removed

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