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Facilitation by substance P and inhibition by (+)-tubocurarine of the 5-HT_3 receptor-mediated Bezold-Jarisch reflex in rats

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Abstract

The influence of substance P 3 (μg/kg) and (+)-tubocurarine (850 μg/kg) on the Bezold-Jarisch reflex in urethane-anaesthetized rats was studied. The Bezold-Jarisch reflex was induced by the 5-HT_3 receptor agonist phenylbiguanide (0.3, 1, 3 and 10 μg/kg i.v.) and by capsaicin (10 μg/kg i.v.). The 5-HT_3 receptor antagonist ondansetron (10 μg/kg) abolished the phenylbiguanide- but not the capsaicin-stimulated bradycardia, indicating that phenylbiguanide and capsaicin act via different trigger mechanisms (5-HT_3 receptor-dependent and -independent, respectively). Substance P significantly potentiated the phenylbiguanide- but not the capsaicin-induced decrease in heart rate. Also, when the phenylbiguanide-induced response was amplified by substance P, it was abolished by ondansetron. (+)-Tubocurarine inhibited the phenylbiguanide-induced bradycardia, but did not affect the capsaicin-stimulated decrease in heart rate. Our results demonstrate that substance P potentiates but (+)-tubocurarine inhibits the 5-HT_3 receptor-mediated Bezold-Jarisch reflex. Both effects are probably due to direct influences of the drugs on the 5-HT_3 receptors on sensory vagal nerves in the heart.

Keywords: 5-HT_3 receptor; Substance P; (+)-Tubocurarine; Bezold-Jarisch reflex; Capsaicin

1. Introduction

5-Hydroxytryptamine (serotonin; 5-HT) exerts its action via multiple classes of 5-HT receptors (for reviews, see Hoyer et al., 1994; Saxena, 1995), among which only the 5-HT_3 receptor is a ligand-gated cation channel (Derkach et al., 1989; Maricq et al., 1991). In the heart, 5-HT_3 receptors are located on vagal afferent nerve fibres (Cohen, 1992). By activating such epicardial/ventricular 5-HT receptors on C fibre afferents (Thoren, 1979), 5-HT induces a transient reflex bradycardia and hypotension known as the Bezold-Jarisch reflex (Fozard, 1984). The Bezold-Jarisch reflex may play a role in the pathophysiology of several clinically important cardiovascular disorders, including myocardial ischaemia and infarction, syncope and heart failure (Mark, 1983).

Experiments in NG 108-15 and N1E-115 cells revealed that responses mediated by 5-HT_3 receptors are potentiated by substance P (Reiser and Hamprecht, 1989; Bönisch et al., 1993; Emerit et al., 1993; Riad et al., 1994; Barann et al., 1995) and that, under certain conditions, substance P given alone may even stimulate these receptors (Reiser and Hamprecht, 1989). In this context, it is of interest that substance P and 5-HT are co-localized in many central neurons (Hökfelt et al., 1980) or in the same neuronal pathway (Pilowsky et al., 1995). On the basis of these findings, the first aim of our study was to examine whether substance P also potentiates the Bezold-Jarisch reflex, i.e. a response mediated by 5-HT_3 receptors on cardiac sensory vagal nerves in anaesthetized rats.

The second aim was to determine the effect of (+)-tubocurarine on the peripheral 5-HT_3 receptors in the heart. In addition to its antagonistic property at post-junctional nicotinic cholinoceptors on the skeletal muscle motor endplate, (+)-tubocurarine has been shown to antagonize 5-HT_3 receptor-mediated responses in mouse central neurons, murine neuroblastoma and neuroblastoma-glioma cells (Peters et al., 1990, Newberry et al., 1991, 1992; Emerit et al., 1993; Gill et al., 1995). However, under in vivo conditions, the effect of (+)-tubocurarine on 5-HT_3 receptors has not yet been examined.

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In order to determine the site of action of substance P and (+)-tubocurarine, additional experiments were performed in which the influence of both substances on the capsaicin-induced Bezold-Jarisch reflex was studied.

2. Materials and methods

Male Wistar rats weighing 180–370 g were anaesthetized with urethane (1.25 g/kg i.p.). The trachea was cannulated and heart rate was derived from the ECG recorded via s.c. electrodes. Diastolic blood pressure was measured from the right carotid artery via a pressor transducer (Gould P23ID). The electrodes for the ECG and the pressor transducer were connected to the monitor Trendscope 8031 (S&W Vickers, Poland). The left femoral vein was cannulated for i.v. injections of drugs administered in a volume of 0.5 ml/kg. Body temperature was kept constant at 37 ± 1°C, using a tungsten lamp and monitored by a rectal probe transducer. After 15–30 min of equilibration, during which the cardiovascular parameters were allowed to stabilize, experiments were performed.

The Bezold-Jarisch reflex (bradycardia) was induced by injection of the 5-HT_3 receptor agonist phenylbiguanide (0.3, 1, 3 and 10 μg/kg) or capsaicin (10 μg/kg; each dose was studied in a separate animal). Injection of phenylbiguanide or capsaicin was repeated every 10 min until the bradycardia in response to three subsequent injections remained constant. 10 min later, one (or in the case of interaction experiments, two) of the following test drugs (or the solvent 0.9% NaCl) was administered: substance P, 3 μg/kg; mastoparan, 3 or 300 μg/kg; ondansetron, 10 μg/kg; (+)-tubocurarine, 850 μg/kg; or pipecuronium, 300 μg/kg. 15 s after administration of substance P or mastoparan and/or 5 min after administration of ondansetron, (+)-tubocurarine or pipecuronium, the bradycardic response to phenylbiguanide or capsaicin was examined. Immediately after administration of (+)-tubocurarine or pipecuronium, rats were artificially ventilated with air (60 strokes/min) by using a respiratory system (Medipan, Poland).

2.1. Calculations and statistics

Results are given as means ± S.E.M. throughout the paper (n, number of rats). The control bradycardia was taken as the last bradycardia induced 10 min before administration of the interacting drugs. The decrease in heart rate is expressed as a percentage of the basal heart rate immediately before injection of phenylbiguanide or capsaicin. For comparison of mean values, the t-test for paired or unpaired data was used. The differences were considered as significant when $P < 0.05$.

2.2. Drugs used

The following drugs were used: phenylbiguanide (1-phenylbiguanide; Aldrich, Germany), substance P, (+)-tubocurarine, capsaicin (8-methyl-*N*-vanilyl-6-nonenamide), mastoparan, urethane (Sigma, USA), ondansetron (ondansetron hydrochloride dihydrate; Glaxo, UK), pipecuronium (pipecuronium bromide; Gedeon, Richter, Hungary). Phenylbiguanide, substance P, mastoparan, (+)-tubocurarine and pipecuronium were dissolved in saline, capsaicin in a mixture of saline and ethanol (15:1) and urethane in water.

3. Results

In the various experimental groups of urethane-anaesthetized rats, basal heart rate and diastolic blood pressure ranged from 313.8 ± 15.9 ($n = 6$) to 416.7 ± 16.7 beats/min ($n = 6$) and from 45.0 ± 3.5 ($n = 5$) to 68.2 ± 4.5 mmHg ($n = 6$), respectively. Intravenous injection of saline or the mixture of saline and ethanol (15:1) in the same volume as used for injection of phenylbiguanide or capsaicin, respectively, did not affect heart rate or blood pressure.

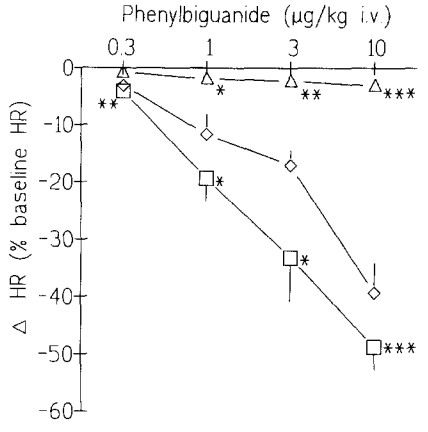

Fig. 1. Influence of substance P and/or ondansetron on the phenylbiguanide-stimulated decrease in heart rate (HR) in urethane-anaesthetized rats. The bradycardia induced by 0.3, 1, 3 or 10 μg/kg phenylbiguanide 10 min before administration of the interacting drug was considered as the control value. The test bradycardia was evoked 15 s after administration of 0.9% NaCl (◇) or substance P (□; 3 μg/kg) and 5 min after injection of ondansetron (△; 10 μg/kg). The basal HR immediately before the phenylbiguanide (0.3, 1, 3 and 10 μg/kg)-induced test bradycardia was 319.5 ± 21.8, 327.0 ± 18.5, 379.8 ± 13.0 and 336.0 ± 19.0 beats/min in the NaCl-treated group, 335.3 ± 10.1, 388.8 ± 22.8, 416.7 ± 16.7 and 375.5 ± 24.1 beats/min in the substance P-treated group and 313.8 ± 15.9, 388.2 ± 19.0, 401.4 ± 26.1 and 358.8 ± 14.9 beats/min in the ondansetron and substance P-treated group, respectively. In many cases S.E.M. is smaller than the symbol. Means ± S.E.M. for 4–10 rats. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to the respective controls.

The i.v. administration of the 5-HT_3 receptor agonist phenylbiguanide 0.3, 1, 3 and 10 μg/kg induced a short-lasting (3–10 s), dose-dependent decrease in heart rate (Fig. 1) and blood pressure (data not shown). The maximal fall in heart rate was about 40% of the basal value immediately before injection of phenylbiguanide 10 μg/kg. The decrease in heart rate was followed by a slight increase (1–5 min). Only the rapid responses in both heart rate and blood pressure are associated with activation of the Bezold-Jarisch reflex (Fozard, 1984). The 5-HT_3 receptor antagonist ondansetron 10 μg/kg, which given alone did not affect heart rate, almost abolished the phenylbiguanide-induced bradycardia (Fig. 2). The i.v. administration of capsaicin 10 μg/kg also reduced heart rate (Fig. 2) and blood pressure (data not shown). Again, the bradycardia was of short duration (5–15 s). The maximal decrease in heart rate was about 35% of the basal value immediately before administration of capsaicin 10 μg/kg.

Substance P 3 μg/kg, mastoparan 3 and 300 μg/kg, (+)-tubocurarine 850 μg/kg and pipecuronium 300 μg/kg (both neuromuscular blocking drugs were equi-effective in paralysing the respiratory muscle) did not induce reflex bradycardia by themselves (results not shown). 15 s after administration, substance P 3 μg/kg negligibly increased basal heart rate [by 6% ($n = 28$, $P < 0.001$) and 7% ($n = 11$, $P < 0.05$) in the phenylbiguanide- and capsaicin-treated groups, respectively]. It slightly diminished basal diastolic blood pressure [by 9% ($n = 28$, $P < 0.01$) and 8% ($n = 11$, $P < 0.01$) in the phenylbiguanide- and capsaicin-treated groups, respectively]. Mastoparan, a peptide toxin from wasp venom that mimics the effect of substance P on G-proteins, at doses of 3 and 300 μg/kg increased basal heart rate even less (by 3%; $n = 6$, $P < 0.01$ and 6%; $n = 5$, $P < 0.01$, respectively) and did not change basal blood pressure. (+)-Tubocurarine 850 μg/kg and pipecuronium 300 μg/kg slightly diminished blood pressure [by 8% ($n = 28$, $P < 0.05$) and 9% ($n = 30$, $P < 0.01$), respectively] and did not affect heart rate.

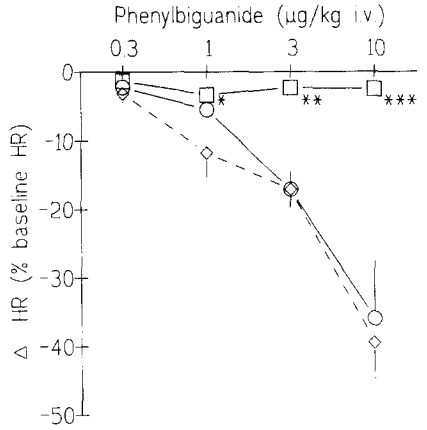

Fig. 3. Influence of (+)-tubocurarine (□: 850 μg/kg) and pipecuronium (○; 300 μg/kg) on the phenylbiguanide-stimulated decrease in heart rate (HR) in urethane-anaesthetized rats artificially ventilated with air; for comparison, the bradycardia in non-ventilated control rats (injection of 0.9% NaCl; for details see legend to Fig. 1; ◇, broken line) is also shown. The bradycardia induced by 0.3, 1, 3 or 10 μg/kg phenylbiguanide 10 min before administration of the interacting drug was considered as the control value. The test bradycardia was evoked 5 min after administration of the interacting drug. The basal HR immediately before the phenylbiguanide (0.3, 1, 3 and 10 μg/kg)-induced test bradycardia was 371.2 ± 17.2, 412.0 ± 13.9, 357.9 ± 18.6 and 358.1 ± 12.3 beats/min in the pipecuronium-treated group and 380.1 ± 18.4, 372.6 ± 31.2, 360.2 ± 35.7 and 373.3 ± 29.7 beats/min in the (+)-tubocurarine-treated group, respectively. In many cases S.E.M. is smaller than the symbol. Means ± S.E.M. for 5–7 rats. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to the respective controls.

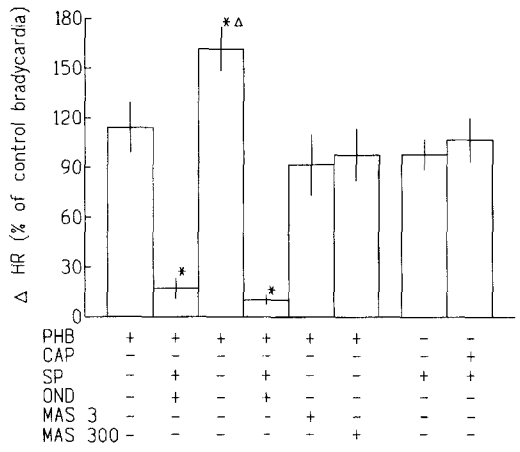

Fig. 2. Influence of substance P, mastoparan and ondansetron on the phenylbiguanide (PHB; 10 μg/kg)-induced or capsaicin (CAP; 10 μg/kg)-induced decrease in heart rate (HR) in urethane-anaesthetized rats. The bradycardia induced by 10 μg/kg PHB or 10 μg/kg CAP 10 min before administration of the interacting drug was considered as the control value. 100% corresponds to a bradycardic response of 102.2 ± 13.6 to 133.8 ± 15.4 beats/min in the various series of control experiments. The test bradycardia was evoked 15 s after administration of substance P (SP; 3 μg/kg) or mastoparan (MAS 3; 3 μg/kg; MAS 300; 300 μg/kg) and 5 min after injection of ondansetron (OND; 10 μg/kg). Means ± S.E.M. for 5–11 rats. * $P < 0.001$ compared to the respective controls, $^{\Delta}P < 0.01$ compared to the respective group of the capsaicin-induced bradycardia.

Substance P 3 μg/kg significantly augmented the bradycardic response elicited by phenylbiguanide 0.3, 1, 3 and 10 μg/kg (Fig. 1) but had no influence on the bradycardia induced by capsaicin 10 μg/kg (Fig. 2). The reflex responses to all doses of phenylbiguanide given in the presence of substance P 3 μg/kg was completely inhibited by ondansetron 10 μg/kg (Fig. 1). Mastoparan 3 or 300 μg/kg did not affect the bradycardia induced by phenylbiguanide 10 μg/kg (Fig. 2).

In order to examine the influence of (+)-tubocurarine and pipecuronium on the reflex bradycardia, rats were artificially ventilated, since at the doses investigated (850 and 300 μg/kg, respectively) both drugs induced apnoea. (+)-Tubocurarine abolished the phenylbiguanide (0.3–10 μg/kg)-induced bradycardia (Figs. 3 and 4) but had no influence on the response to capsaicin (10 μg/kg; Fig. 4),

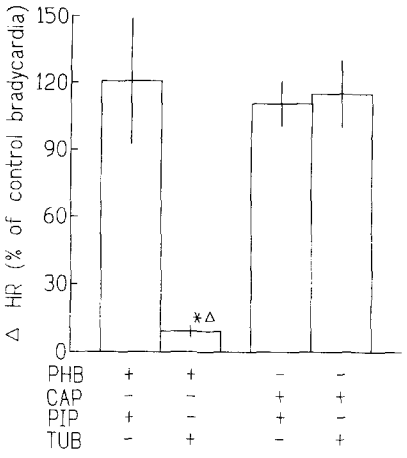

Fig. 4. Influence of (+)-tubocurarine (TUB; 850 μg/kg) and pipecuronium (PIP; 300 μg/kg) on the phenylbiguanide (PHB; 10 μg/kg)- or capsaicin (CAP; 10 μg/kg)-induced decrease in heart rate (HR) in urethane-anaesthetized rats. The bradycardia induced by 10 μg/kg PHB or 10 μg/kg CAP 10 min before administration of the interacting drug was considered as control value. 100% corresponds to a bradycardic response of 97.2 ± 14.3 to 115.5 ± 7.4 beats/min in the various series of control experiments. The test bradycardia was evoked 5 min after administration of the interacting drug. Means ± S.E.M. for 6–8 rats. $^{***}P < 0.001$ compared to the respective controls, $^{\Delta}P < 0.001$ compared to the respective group of the capsaicin-induced bradycardia.

whereas pipecuronium did not change the bradycardia evoked by both phenylbiguanide (0.3–10 μg/kg; Figs. 3 and 4) and by capsaicin (10 μg/kg; Fig. 4).

4. Discussion

The present study was designed to investigate the influence of substance P and (+)-tubocurarine on cardiac 5-HT_3 receptors by using the Bezold-Jarisch reflex as a functional model. To confirm the involvement of such receptors in this reflex (Butler et al., 1988; Bogle et al., 1990; Cohen, 1992) under the present conditions, the bradycardia induced by the 5-HT_3 receptor agonist phenylbiguanide was abolished by the selective 5-HT_3 receptor antagonist ondansetron. In agreement with previous results obtained in vitro (for references, see Section 1), we found here that substance P potentiated and (+)-tubocurarine inhibited the phenylbiguanide-induced Bezold-Jarisch reflex. However, in our in vivo experiments, not only the 5-HT_3 receptors on the afferent cardiac nerve fibres have to be considered as sites of action of both substances but also the central pathway mediating the reflex (a very improbable possibility in view of the physicochemical properties of the peptide substance P and of (+)-tubocurarine), the efferent vagal fibres and the cardiac myocytes.

Experiments in which capsaicin was used to evoke the Bezold-Jarisch reflex were carried out to exclude possibilities other than 5-HT_3 receptors as the sites of action of substance P and (+)-tubocurarine. Capsaicin resembles 5-HT_3 receptor agonists in that it also stimulates reflex bradycardia through activation of the unmyelinated C and small-diameter afferent fibres of the heart (Thoren, 1979), but the 5-HT_3 receptor antagonist ondansetron was found not to affect the capsaicin-stimulated bradycardia (Malinowska et al., 1995). Accordingly, 5-HT_3 receptors are not involved in the capsaicin-induced Bezold-Jarisch reflex, but all other components of the reflex subsequent to its initiation in the afferent cardiac nerve fibres are identical. Thus, the failure of substance P to amplify, and (+)-tubocurarine to inhibit, the Bezold-Jarisch reflex when induced by capsaicin (in view of the ability of these drugs to modify the reflex when evoked by phenylbiguanide) rules out all sites of action mentioned above with the exception of the 5-HT_3 receptors on the cardiac afferent nerves.

The involvement of nicotinic cholinoceptors or effects related to artificial respiration in the inhibitory action of (+)-tubocurarine on the phenylbiguanide-induced Bezold-Jarisch reflex is also excluded by our experiments with pipecuronium at a dose which produced relaxation of respiratory muscle; this drug is another non-depolarising neuromuscular blocking agent which, however, had no influence on either the phenylbiguanide- or the capsaicin-stimulated Bezold-Jarisch reflex. Our results with the neuromuscular blocking drugs are compatible with findings reported in the literature, viz. that vecuronium and artificial respiration do not inhibit the 5-HT-induced Bezold-Jarisch reflex in rats (Saito et al., 1994) and that (+)-tubocurarine does not affect a cardiovascular reflex in which 5-HT_3 receptors are not involved, i.e. the phenylephrine-stimulated baroreflex in rats (El-Mas and Abdel-Rahman, 1993). Since under in vivo conditions we were able to examine only one dose of (+)-tubocurarine which completely blocked spontaneous respiration, the mechanism underlying its inhibitory effect on the phenylbiguanide-induced Bezold-Jarisch reflex can only be derived from the results of previous in vitro studies. Newberry et al. (1992) reported that (+)-tubocurarine antagonized 5-HT_3 receptor-mediated responses on the rat vagus in a competitive manner with an IC_{50} of 0.3–1.0 μmol/l. Analogously, in our in vivo model, (+)-tubocurarine may be assumed to act as a competitive antagonist at the 5-HT_3 receptors on the sensory vagal nerve fibres in the heart, which are activated by phenylbiguanide.

Substance P belongs to a structurally related family of bioactive peptides known as tachykinins which act through three receptor types, NK_1, NK_2 and NK_3. The three tachykinin receptors have been cloned, sequenced and shown to be members of the G-protein-linked superfamily of metabotrobic receptors (for a review, see Regoli et al., 1994). In our experiments, we observed three different effects of substance P, viz. a slight decrease in blood pressure, a weak increase in heart rate and a clear-cut

potentiation of the phenylbiguanide-induced reflex bradycardia. On the basis of results reported in the literature, the vasodepressor and tachycardic response to substance P may be assumed to be mediated by NK_1 and NK_2 receptors, respectively (for a review, see Regoli et al., 1994). However, for the enhancement of the Bezold-Jarisch reflex, no evidence for such a mechanism is available. In particular, it can be excluded that this potentiating effect of substance P is a consequence of the changes in the basal cardiovascular parameters, since no potentiation was observed when the Bezold-Jarisch reflex was evoked by capsaicin although, also in these experiments, substance P itself produced slight hypotension and a positive chronotropic effect.

The in vitro experiments with substance P in NG 108-15 and N1E-115 cells, quoted in the Introduction, revealed that the concentrations of this peptide necessary to induce potentiation of 5-HT_3 receptor activation are in the micromolar range and, thus, much higher than those required to activate NK receptors. Moreover, the potency of substance P and some of its analogues does not fit to the pharmacological characteristics of tachykinin receptors (Reiser and Hamprecht, 1988; Emerit et al., 1993). In addition to its activation via NK receptors, substance P has been shown to be capable of directly activating G-proteins in certain cells (Mousli et al., 1990). In order to exclude such a receptor-independent mechanism in the potentiation by substance P of the effect of 5-HT_3 receptor agonists, experiments were carried out with mastoparan. This peptide from wasp venom shares the property of substance P to activate G-proteins directly (Mousli et al., 1990). However, in NG 108-15 cells, mastoparan did not affect the cation influx induced by 5-HT_3 receptor activation (Emerit et al., 1993). Analogously, we found in our in vivo model that mastoparan, even when injected at a very high dose, did not increase the phenylbiguanide-induced reflex bradycardia. These results with mastoparan obtained in vitro and in vivo argue against the possibility that a direct activation of G-proteins plays a role in the potentiation by substance P of the effects of the 5-HT_3 receptor agonist. However, a plausible explanation would be that substance P acts at an allosteric modulatory site of the 5-HT_3 receptor, thus, producing an increase in cation flux through this channel, e.g. by affecting its open frequency or duration, without necessarily influencing its ligand recognition sites. This conclusion is compatible with the findings that substance P by itself did not induce reflex bradycardia and that the 5-HT_3 receptor antagonist ondansetron abolished the response to phenylbiguanide not only when it was given alone but also when it was reinforced by substance P.

In conclusion, the present study revealed that substance P potentiates and (+)-tubocurarine inhibits the phenylbiguanide-induced Bezold-Jarisch reflex in urethane-anaesthetized rats, probably by directly influencing the 5-HT_3 receptors on the vagal afferent nerve fibres to the heart.

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