ed plants from day 30 onwards correlated with the infection of the root system. Histochemical study suggested that the higher amounts of phenol in mycorrhizal plants might be due to deposition of phenols in the fungal structures. Arbuscules, and inter- and intracellular hyphae showed deeply stained phenol deposition compared to cortical cells devoid of the fungus (fig., a, b).

Working with tomato, Dehne and Schönbeck[7] also found that mycorrhizal plants contained higher amounts of phenol compared to uninoculated plants. Initial increase in phenol in mycorrhizal plants is probably due to an incompatible reaction of the host plant to the invading fungus. Similar situations exist when pathogenic fungi invade host plants[14]. Some workers have correlated the presence of phenolics in plants with resistance to pathogens[15]. Mycorrhizal inoculation is known to impart resistance to the host against disease[16]. Another study conducted by us indicated that the concentration of O–D phenol present in mycorrhizal roots inhibited in vitro growth of the root pathogen, *Sclerotium rolfsii*[17]. The reduction in dry weight of mycelium grown in potato dextrose broth for 15 days was 27%. Thus, it seems that the higher amount of phenols might be one of the factors responsible for increased disease resistance found in mycorrhizal plants.


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## Development of a new tachykinin antagonist

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*Summary.* The mouse urinary bladder possesses a tachykinin receptor which responds to kassinin and eledoisin, but not to some other tachykinins. The action of kassinin, but not that of eledoisin, was blocked in a surmountable manner by a new tachykinin antagonist, D-Pro$^4$, Val$^8$, D-Trp$^{7,9,10}$ (SP)$^{4-11}$.

Within the last 18 months several substance P (SP) antagonists have been described[2–4]. Although these compounds effectively antagonize SP action on a variety of tissues, several problems are associated with their use; they generally exhibit low $pA_2$-values, some exert agonistic actions[5,6], an unsurmountable rather than a surmountable inhibition may be observed and they do not antagonize the actions of SP and some other tachykinins on all tissues.

In the present study we have attempted to develop an antagonist for a tachykinin receptor that is highly selective for 2 members of the tachykinin family of peptides: eledoisin (ED) and kassinin (KA). Although there are many structural differences between these 2 peptides and SP (table), we chose to modify position 8 of the antagonist D-Pro$^4$, D-Trp$^{7,9,10}$ (SP) 4–11, recently described by Mizrahi and coworkers[4]. KA and ED have nonpolar amino acids in position 8; valine and isoleucine, respectively, whereas SP has an aromatic residue, phenylalanine, in this position. The antagonistic properties of these compounds are due to a collection of D-enantomiers at positions 7, 9 and 10, with the amino acid in position 8 being important for binding to the receptor[7].

*Material and methods.* The actions of the tachykinins, in the presence and the absence of tachykinin antagonists, were tested on strips (2 × 10 mm) of the mouse urinary bladder (Swiss White strain). The tissues were suspended in an 85 µl tissue bath[8] and superfused with oxygenated Krebs solution of the following composition (mM): NaCl 118.7, KCl 4.7,

Structures of several tachykinins and 2 tachykinin antagonists

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Substance P | | Arg- | Pro- | Lys- | Pro- | Gln- | Gln- | Phe- | Phe- | Gly- | Leu- | Met-$NH_2$ |
| Physalaemin | | Glp-* | Ala- | Asp- | Pro- | Asn- | Lys- | Phe- | Tyr- | Gly- | Leu- | Met-$NH_2$ |
| Eledoisin | | Glp- | Pro- | Ser- | Lys- | Asp- | Ala- | Phe- | Ile- | Gly- | Leu- | Met-$NH_2$ |
| Kassinin | Asp- | Val- | Pro- | Lys- | Ser- | Asp- | Gln- | Phe- | Val- | Gly- | Leu- | Met-$NH_2$ |
| Compound I | | | | | pro-** | Gln- | Gln- | trp- | Phe- | trp- | trp- | Met-$NH_2$ |
| Compound II | | | | | pro- | Gln- | Gln- | trp- | Val- | trp- | trp- | Met-$NH_2$ |

* Glp, pyroglutamic acid; ** D-amino acids are written entirely in small letters.

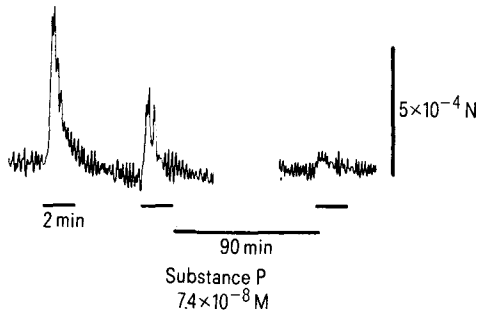


Figure 1. Substance P (SP)-induced contractions of the mouse urinary bladder fade off rapidly (1st contraction) and the SP receptor apparently desensitizes since a subsequent addition of the peptide produces a smaller contractile response (2nd contraction). Receptor desensitization is then nearly complete as a subsequent addition of SP, after 90 min of continuous washout, produces a very small contraction.

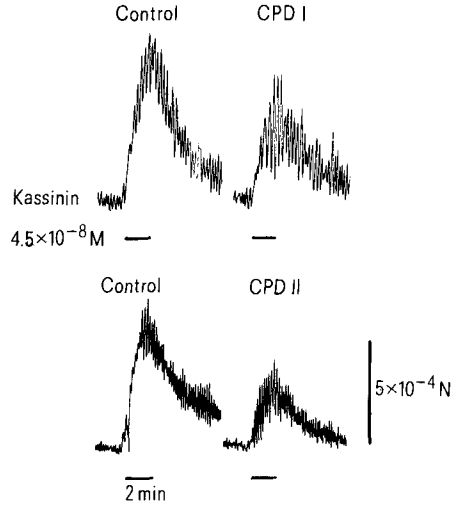


Figure 2. Kassinin produces a strong contraction of the mouse urinary bladder which requires many minutes to regain the baseline even with continuous washout. Two tachykinin antagonists, D-Pro$^4$, D-Trp$^{7,9,10}$ (SP) 4–11 (CPD I), and D-Pro$^4$, Val$^8$, D-Trp$^{9,10,11}$ (SP) 4–11 (CPD II), antagonize the kassinin-induced increase in myogenic activity.

$CaCl_2$ 2.8, $KH_2PO_4$ 1.2, $NaHCO_3$ 15.5, $MgCl_2$ 1.2, glucose 10.1. The physiological solution was bubbled with 95% $O_2$–5% $CO_2$ and maintained at 35 °C. All peptides were prepared from stock solutions of 1 mg/ml in 0.25% acetic acid and were superfused onto the bladder strips at their final concentration. Antagonists were superfused onto the tissues for at least 10 min prior to testing an agonist. The changes in tension were measured isometrically (Grass FT.03C transducers) and the amplified signals were integrated electronically. The integrated force of the 2nd min of a drug-induced contraction was used to construct dose-response curves. From the dose-response curves, obtained in the presence and absence of the antagonist, dose-ratios were calculated.

*Results and discussion.* The mouse urinary bladder responded in a rapid, but rapidly fading manner to SP (fig. 1) and physalaemin (PH). Upon addition of one of these tachykinins at a concentration of $7.4\times10^{-8}$ M, the contractile response returned to the baseline within 1 min and the tissues remained unresponsive for up to 4 h to both SP and PH. In contrast, repetitive non-fading responses to KA and ED were obtained. The $pD_2$-values were $6.94\pm0.06$ for KA and $6.64\pm0.07$ for ED. The actions of these 2 tachykinins were examined in the presence of 2 tachykinin antagonists ($10^{-6}$ M); the D-Pro$^4$, D-Trp$^{7,9,10}$ (SP) 4–11 (compound I) and D-Pro$^4$, Val$^8$, D-Trp$^{7,9,10}$ (SP) 4–11 (compound II; see the table). The dose-response curves to ED were not modified by these 2 antagonists; dose-ratios were $1.46\pm0.15$ and $1.56\pm0.22$ for compounds I and II, respectively. The contractions induced by KA, however, were markedly attenuated by both antagonists (fig. 2). The inhibition due to compound I was of the unsurmountable type, whereas the dose-response curve to KA in the presence of compound II was displaced to the right ($pA_2=6.29\pm0.08$; see fig. 3). The efficacy of KA, however, was not modified by compound II.

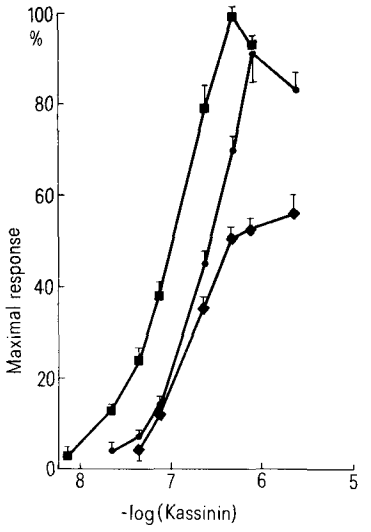


Figure 3. Dose-response curves for kassinin alone (■——■) on the mouse urinary bladder, and kassinin in the presence of 2 tachykinin antagonists: D-Pro$^4$, D-Trp$^{7,9,10}$ (SP) 4–11) (◆——◆), and D-Pro$^4$, Val$^8$, D-Trp$^{7,9,10}$ (SP) 4–11 (●——●).

Lee and coworkers[9] have suggested that SP receptors be subdivided into 2 subtypes: the 'SP-P' for substance P and physalaemin and the 'SP-E' for substance P and eledoisin. A tissue possessing the 'SP-P' receptor subtype would have an order of potency to the tachykinins of PH ≈ SP > ED ≈ KA, and the 'SP-E' receptor a rank order of ED ≈ KA > PH ≈ SP. The mouse urinary bladder would appear to possess a predominant 'SP-E' receptor, but a 2nd tachykinin receptor, which exhibits a tachyphylactic response to SP and PH may exist. The guinea-pig urinary bladder is also thought to possess these 2 types of tachykinin receptors[10].

Although an antagonist which effectively antagonizes both ED and KA remains to be described, the present study suggests that appropriate amino acid substitutions in the recently described SP antagonists may result in compounds with potent and selective antagonistic actions on the 'SP-E' receptor.

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## Effects of bromocriptine on plasma testosterone and gonadotropin levels and testicular lipid fractions in adult rats

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*Summary.* Bromocriptine treatment of adult male rats resulted in a decrease in testicular testosterone (T) content and a reduction in plasma T levels. This was accompanied by increase in testicular total lipids and cholesterol and depletion of testicular phospholipids.

Administration of bromocriptine (CB-154), a dopaminergic agonist capable of suppressing prolactin (PRL) synthesis and release from pituitary acidophil cells, effectively decreases peripheral PRL levels in almost all species thus far investigated[2,3]. In rats, bromocriptine also influences the release of other gonadotropins[4] and a decreasing trend in peripheral testosterone (T) levels was observed in rats and mice injected with either CB-154 or with ergot alkaloids having similar biological activity[5]. Suppression of plasma T levels in these experiments could probably be explained by the CB-154-induced inhibition of PRL release[6] however, CB-154 appears to exert direct effects on Leydig cell function as well. Addition of CB-154 to the incubation media has been shown to inhibit T production by rat testicular cells in vitro[7], while lower concentrations of CB-154 stimulate basal T production by decapsulated mouse testes in vitro[8].

The mechanism(s) of bromocriptine action on testicular steroidogenesis remains to be elucidated; therefore, it was of interest to investigate the effects of bromocriptine injections on testicular lipid fractions, which include precursors for steroidogenesis.

Intact adult male Wistar rats (325 ± 10 g b.wt, 75 days of age) were purchased from Charles River Breeding Laboratories and maintained in a room with controlled illumination (14 h L: 10 h D) and temperature 24 ± 2 °C, with free access to food and water. Six rats received daily s.c. injections of CB-154 (Sandoz Pharmaceuticals, East Hanover, NJ; 1.0 mg in 0.1 ml sesame oil), while 6 rats received vehicle only. 24 h after the last injection, the animals were decapitated while under light ether anesthesia, and trunk blood was collected for plasma hormone assay. The testes were immediately removed, snap frozen and kept at −70 °C until further biochemical analysis. Epididymides, seminal vesicles and ventral prostates were removed and weighed. Plasma levels of LH, FSH, PRL and T and testicular levels of T were measured by radioimmunoassays described previously[8,9]. Testicular total lipids were extracted into Folch medium[10] and estimated colorimetrically according to the method of Frings et al.[11]. Phospholipids in the testis were estimated by the method described previously[12], by measuring the liberated inorganic phosphorus colorimetrically[13]. Free and esterified cholesterol concentrations were estimated colorimetrically after chromatographic separation of these 2 fractions[14].

In CB-154-treated animals, plasma PRL levels were suppressed to negligible values (table 1) in agreement with earlier observations[2,3,9]. Testicular weight seemed slightly reduced and plasma LH and FSH levels elevated, but these apparent differences were not statistically significant. The concentration of T in plasma and testes as well as the total testicular T content was significantly reduced in CB-154-injected rats (tables 1 and 2). The concentration and content of testicular phospholipids were decreased in the animals receiving CB-154 (table 2), while total lipid content of the testes and testicular cholesterol ester levels were increased with no alteration in free cholesterol. A significant reduction in plasma T levels and an apparent increase in plasma LH levels in the present study are reminiscent of the results of Boyns et al.[4] who reported a significant increase in plasma LH levels with simultaneous decrease in plasma T in bromocriptine-treated rats. The reduction in peripheral and testicular T levels probably resulted from PRL deficiency, since PRL increases the number of testicular LH receptors and potentiates LH action on the testis[6,15]. In this context, it is interesting to note the changes in testicular precursors of steroidogenesis after bromocriptine treatment. In hypophysectomized animals, combined deficiency of PRL and gonadotropins is accompanied by accumulation of lipids in seminiferous tubules[16,17] and alterations in testicular phopholipids[18]. In animals with isolated PRL deficiency in the present study, accumulation of cholesterol esters suggests that the activity of enzymes involved in the conversion of esterified cholesterol to T was inhibited. This could have been due to either direct effects of bromocriptine on the testes or to inhibition of PRL release with consequent reduction in the responsiveness of the testis to LH.

Table 1. Effects of bromocriptine (CB-154) injections on plasma prolactin (PRL), gonadotropin and testosterone (T) levels in mature male rats (means ± SD)

| Hormone (ng/ml) | CB-154-treated (6) | Control (6) | Difference (%) |
|---|---|---|---|
| PRL | 1.0 ± 0.2 | 49.0 ± 8.7 | − 98* |
| T | 2.98 ± 1.10 | 4.75 ± 0.97 | − 37** |
| FSH | 263 ± 84 | 231 ± 53 | + 14 |
| LH | 17 ± 12 | 12 ± 4 | + 40 |

*Significant at 0.1% level; **significant at 1% level.