Structure-activity relationships of bradykinin potentiating peptides

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In order to establish the nature of the active group(s) or structural characteristics of certain bradykinin potentiating pentapeptides, a number of A-VI-5 (Val-Glu-Ser-Ser-Lys) analogues and fragments were synthetized and tested for bradykinin potentiating activity (Ufkes *et al.*, 1978). It was concluded that (1) the polar groups of the side-chains are not essential; (2) the chain-length (at least 5 amino acids) and the lipophilicity are of much more importance; (3) the free N-terminal NH_2-group is not essential; (4) aromatic amino acids in position 3 result in highly active peptides.

A further study was made using peptides with a more 'neutral' character (Ala-Ala-Ala-Ala-Ala or Val-Ala-Ala-Ala-Ala) and substituted with Tyr or Trp in position 3. In order to increase the hydrophilicity of these highly insoluble peptides Lys was introduced. To confirm the importance of the presence of an aromatic amino acid in position 3, Trp was also substituted in position 2 and 4.

Several BPP_{5a}-(Pyr-Lys-Trp-Ala-Pro) analogues were synthetized and tested for bradykinin potentiating activity. Trp was replaced by Phe or (D)-Trp in position 3. To determine the importance of position 1 and 2 Pyr and Lys were replaced as well.

The synthesis, the purification as well as the determination of the bradykinin potentiating were performed as previously described (Ufkes *et al.*, 1978).

From these findings it can be concluded that (1) a certain balance between lipophilicity and hydrophilicity is required; (2) an (L)-aromatic amino acid exclusively in position 3 is essential for a high activity; (3) among the aromatic amino acids (L)-tryptophan is the best choice; (4) the amino acids in positions 1 and 2 in BPP_{5a} are responsible for additional activity; (5) Pyr-Lys-Trp-Ala-Pro seems to be the optimal configuration for a bradykinin potentiating pentapeptide.

Reference

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Antioestrogenic derivatives of nafoxidine stimulate progesterone receptor synthesis *in vivo*

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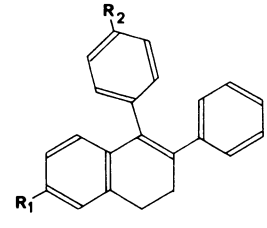
Nafoxidine (U 11,100A) is a non-steroidal antioestrogen that has been used to treat breast cancer (Legha, Slavik & Carter, 1976), and in the laboratory has been used for the investigation of antioestrogenic mechanisms. Both *in vivo* and *in vitro* oestradiol and the antioestrogen tamoxifen, which is a partial oestrogen agonist, stimulate progesterone receptor synthesis, whilst *in vitro*, nafoxidine is ineffective (Horwitz, Koseki & McGuire, 1978; Koseki, Zava, Chamness & McGuire, 1977). It is possible that the progesterone receptor synthesis promoted by tamoxifen requires conversion into the fully oestrogenic *cis* geometrical isomer, whereas the rigid structure of nafoxidine will not permit this isomerisation. We have therefore determined whether nafoxidine-like antioestrogens (U 22410A and U 10,520, Figure 1) can stimulate progesterone receptor synthesis *in vivo*.

U 22410A and U 10,520 are both partial oestrogen agonists with antioestrogenic properties in the 3d immature rat (Alderley Park strain) uterine weight test, and both increase the uterine binding of the synthetic progestogen [^{3}H]-R5020 (17α,21-Dimethyl-19 norpregna-4,9 diene-3,20-dione [17αMe^{3}H]) using the method described by Vu Hai & Milgrom (1977).



	R_1	R_2	
U-11,100A (nafoxidine)	OCH_3	OCH_2CH_2N	hydrochloride
U-22,410A	H	OCH_2CH_2N	hydrochloride
U-10,520	OCH_3	$OCH_2CH_2N(C_2H_5)_2$	

Figure 1

In a separate experiment U 10,520 (100 μg in 0.1 ml arachis oil) was administered s.c. to immature female rats, and groups of 10 were sacrificed 8, 14, 22, 30, 46 and 66 h later. By 14 h [^{3}H]-R5020 binding increased from 1.03 ± 0.15 pmol/uterus (controls) to 5.22 ± 0.04 pmol/uterus. The administration of cycloheximide (5 μg in 0.1 ml 0.9% saline s.c. 2 hourly) for 6 h before and 16 h after U 10,520 produced a 16 h delay in the increase in [^{3}H]-R5020 binding.

Experiments were also undertaken to determine the ligand specificity of [^{3}H]-R5020 binding in 100,000 *g* supernatants derived from oestradiol or U 10,520 stimulated uteri.

Progesterone and norethindrone produced concentration-dependent (1×10^{-8}–3.3×10^{-7} M) inhibition of [^{3}H]-R5020 (1×10^{-8} M) binding, whereas testosterone, triamcinolone and cortisol were without effect up to 3.3×10^{-7} M for both cytosols.

In conclusion, nafoxidine-like antioestrogens stimulate progesterone receptor synthesis *in vivo*, demonstrating that an ability to be isomerised to a more oestrogenic configuration is not a pre-requisite for activity. However, these studies have not excluded the possibility that other metabolites of these antioestrogens may initiate the progesterone receptor synthesis.

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Interactions between chlordiazepoxide and food deprivation determining choice in a food-preference test

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Rolls & Rolls (1973) introduced a form of food-preference test in which food-deprived rats are given a choice between familiar laboratory chow and a range of palatable, *novel* foods. Bilateral lesions of the basolateral amygdala appear to overcome food neophobia, since unlike control animals, lesioned animals spend much more time eating the novel foods than the familiar chow (Box & Mogenson, 1975; Rolls & Rolls, 1973). Pharmacological treatment can mimic the amygdala lesion effect, since spiperone also increases the choice of novel foods (Cooper, Sweeney & Toates, 1979). In contrast, chlordiazepoxide and diazepam, at relatively low doses, do not exhibit an antineophobic action, as proposed by Poschel (1971); instead, they increase the time spent eating familiar food, whilst leaving the response to novel foods relatively unchanged (Cooper & Crummy, 1978; Cooper, Crummy & Skan, 1977; Cooper & Francis, 1979). The benzodiazepine action is consistent with a direct stimulation of appetite.

In the present experiment, each rat was observed for 600 s in a box which had 6 food containers equally spaced on the grid floor. The 6 foods were familiar chow pellets, and a range of novel foods: apple, chocolate, biscuit, carrot, cheese, Sugar Puffs. The rat's behaviour was monitored on a CCTV system. Each test session was recorded at 2 frames per second, and subsequently each frame was scored according to several behavioural categories. These included eating food (coded according to each food variety), contact with food (similiarly coded), locomotion, rearing, sniffing (head and vibrissae movement without locomotion), and grooming. A computer program converted the frame analysis into durations for each behavioural category. The subjects were 48 adult male Lister rats, half were tested 22 h food-deprived and half were 3 h food-deprived. At each deprivation level, the animals were assigned to 4 injection groups: chlordiazepoxide HCl at 2.5, 5.0 and 10.0 mg/kg, and saline as a control injection. All injections were given i.p., 30 min before the test period. All feeding tests were run at the start of the dark phase of the daily dark-light cycle.

In the saline-treated groups, the 22 h food-deprived rats had shorter latencies to begin feeding ($t = 3.06$, 10 d.f., $P < 0.01$), and spent considerably longer eating familiar chow ($t = 4.10$, $P < 0.002$) compared with the 3 h food-deprived rats. Chow eating times were 218.2 ± 20.1 s (mean ± s.e. mean) and 69.8 ± 15.9 s respectively. In contrast, increasing the level of food deprivation did not significantly affect the time spent eating novel foods. The more deprived animals spent less time engaged in sniffing behaviour than the less deprived animals ($t = 5.48$, $P < 0.0003$),