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## ABSTRACT

## BACKGROUND

>Melanotan II (MT-II) is a cyclic synthetic peptide analog of  $\alpha$ -MSH with an agonist activity at 4 of the 5 known melanocortin receptors: MC1R, MC3R, MC4R, MC5R.

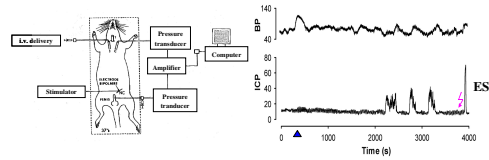
>Melanotan II is a potent initiator of erections in men with psychogenic and organic erectile dysfunctions (Wessels et al., 2000).

>MT-II induces penile erection in conscious and anesthetized rats (Wessels et al., 2003a;2003b).

## METHODS

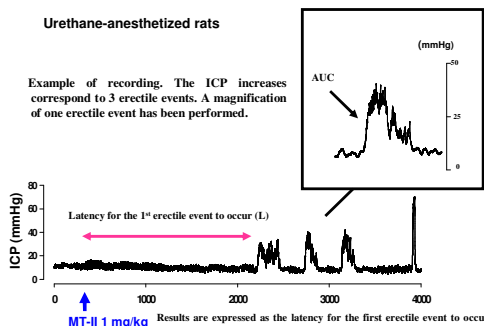
### Evaluation of the initiator activity of MT-II on erectile function in urethane-anesthetized rats

- > Urethane-anesthetized male Sprague-Dawley rats (225-250 g).
- > Evaluation of the erectile response by monitoring of the intracavernous pressure (ICP) after MT-II (0.1, 0.3 and 1 mg/kg) or vehicle (saline) injections in the jugular vein (i.v.).
- > Electrical stimulation (ES) of the cavernous nerve (30 s overall duration, 6V, 1 ms pulse) was performed at the end of experiment to certify correct implantation of intracavernosal catheter.
- > ICP was recorded for 60 minutes post injection (▲).



### Urethane-anesthetized rats

Example of recording. The ICP increases correspond to 3 erectile events. A magnification of one erectile event has been performed.



Results are expressed as the latency for the first erectile event to occur (L), the number of erectile events (N) and the area under the curve of the erectile events (AUC). L and N were averaged per group of rats. AUC was averaged per rat and then per group of rats.

## OBJECTIVES

>To investigate the proerectile activity of MT-II in anesthetized and conscious rats.

>To assess whether anesthesia could modify the proerectile activity of MT-II, by comparing measures in conscious and anesthetized rats.

### Evaluation of the initiator activity of MT-II on erectile function in conscious rats

- > Male Sprague-Dawley rats (225-250 g).
- > Telemetric recording was performed according to Bernabé et al. (Am J Physiol, 1999). Rats were implanted with a telemetric device (C40, Data Sciences, St Paul, MN, USA) (Figure1) allowing measurement of ICP after MT-II (0.1, 0.3 and 1 mg/kg) or vehicle (saline) injections in the vein (i.v.) of the tail.
- > Insertion of the tip of the recording catheter into the proximal shaft of the right corpus cavernosum (Figure 2). The implanted transmitter was placed subcutaneously at the lateral aspect of the abdominal wall (Figure 3).
- > Erectile episodes were visually identified and scored by a trained observer, when the male rat stood up on the extremity of its hindlimbs, bent its head toward the genital region, with the penis emerged from its sheath, and displayed hip movements (Sachs et al., 1994).
- > ICP was recorded for 90 minutes post injection.

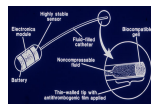


Figure 1

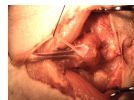


Figure 2

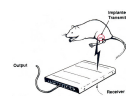
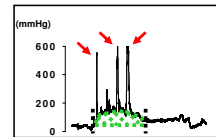


Figure 3

### Conscious rats

Example of recording. Each ICP increase corresponds to an erectile episode behaviorally scored by a trained observer. A magnification of one erectile episode has been performed.



Results are expressed as the latency for the first erectile episode to occur (L), the number of erectile episodes (N) and the area under the curve of the erectile episodes (AUC<sub>tot</sub>). L and N were averaged per group of rats. AUC<sub>tot</sub> and AUC<sub>int</sub> were averaged per rat and then per group of rats.

> The plateau phase, delimited by the dotted green lines, corresponds to the autonomic component of penile erection, i.e. engorgement of the corpora with blood, due to relaxation of the smooth muscles and subsequent increase in intracavernous pressure. Corresponding area under the curve is called AUC<sub>int</sub>.

> The phasic increases of the ICP (red arrows) are caused by contractions of the ischiocavernosus and bulbospongiosus muscles (striated muscles located at the penile crus). Contraction of the ischiocavernosus muscles squeeze the blood trapped in the corpora cavernosa and thus result in further increase in ICP.

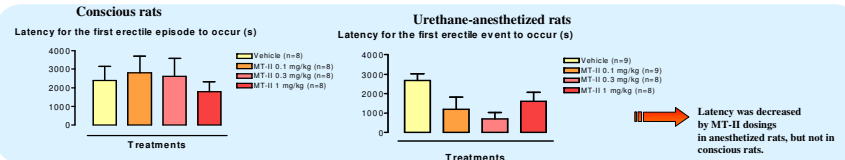
> Total area under the curve of the whole ICP increase (AUC<sub>tot</sub>) corresponds to the total area of the peaks superimposed on the plateau phase.

## SUMMARY OF RESULTS

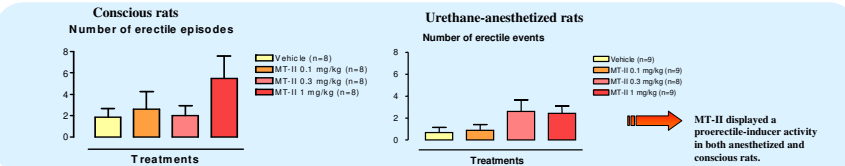
> A proerectile inducer activity of MT-II was measured both in urethane-anesthetized and conscious rats, even if statistically non significant.

> ICP increases during erectile events/episodes have been recorded during 60 min in urethane-anesthetized rats and during 90 min in conscious rats. The number of erectile episodes/events was in the same range when comparing anesthetized with conscious rats, even if MT-II at the dosing of 1 mg/kg displayed a higher number of erectile episodes in conscious rats.

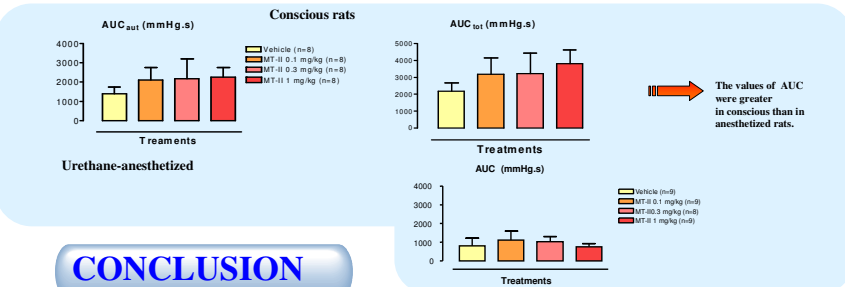
## RESULTS



Latency was decreased by MT-II dosing in anesthetized rats, but not in conscious rats.



MT-II displayed a proerectile-inducer activity in both anesthetized and conscious rats.



The values of AUC were greater in conscious than in anesthetized rats.

## CONCLUSION

> MT-II at the highest dosing (1 mg/kg) displayed a proerectile-inducer activity in both urethane-anesthetized and conscious rats. Both techniques appear to be robust and reproducible.

> Urethane, known to modestly affect multiple neurotransmitter systems at an anesthetic concentration (Hara and Harris, 2002), did not impair the initiator effect on erectile events exerted by MT-II.

> Anesthetized rat appear to be a reliable model to screen CNS acting drugs for their initiator and facilitator effect (#1618) on penile erections.

> Further experiments performed in conscious animals using telemetry allow drug investigations in more physiological conditions.

## References

- > Hara K and Harris RA (2002). The anesthetic mechanism of urethane: the effects on neurotransmitter-gated ion channels. *Anesth Analg* 94 (2):313-318.
- > Sachs B D, Akasofu K, Citron JH, Daniels SB, Nator JH (1994). *Physiol Behav* 51(5): 1073-1075.
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- > Wessels H, Hruby VJ, Hestley J, Han G, Balas-Schroeder P, Francour TW (2003). Acetylcholinesterase-DPho-Ag-Tyr-Lys-NH2 induces penile erection via brain and spinal melanocortin receptors. *Neuroscience* 2003;118(3):755-762.

	Latency (s)			
mean±SEM	Vehicle	0.1 mg/kg	0.3 mg/kg	1 mg/kg
Anesthetized	3075±350	1194±63	702±115	591±242
Conscious	2410±729	2812±909	2617±954	1780±556
	Number of erectile events			
Anesthetized	0.7±0.5	0.9±0.5	2.6±1.1	2.4±0.7
Conscious	1.9±0.8	2.6±1.6	2.0±0.9	3.5±2.1
	AUC (mmHg.s)			
Anesthetized	811±416	1108±484	1033±273	750±179
Conscious	1404±360	215±63	2177±1018	226±480