HOW TO CORROBORATE A SPINAL MECHANISM OF ACTION FOR A DRUG ? EXAMPLE OF THE HYPOTENSIVE EFFECT OF BROMOCRIPTINE IN THE RAT

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***Résumé** : les effets hypotenseurs de la Bromocriptine sont médiés en partie chez le rat par la stimulation des récepteurs dopaminergiques spinaux. Après une transection complète de la moëlle épinière, ces effets hypotenseurs sont accrus et cet accroissement semble lié à une hypersensibilité spinale.*

***Abstract** : Bromocriptine - induced hypotensive effects are induced partly by the stimulation of spinal dopamine receptors. After a complete spinal transection, these hypotensive effects are increased and this increase seems to be induced by a spinal hypersensitivity mechanism.*

A great, and rapidly increasing, number of neuromediators are shown to be present in the spinal cord and are involved in numerous functions. Therefore, their anatomic and functional studies are growing, as the possibilities for hypothesizing a spinal mechanism of action for a drug, directly dependent on the demonstration of the presence in spinal cord of the biological system the drug is acting upon.

To suggest such a spinal mechanism, different methods can be used, and specially the direct administration of the drug in the spinal cord with a catheter inserted in the subarachnoidal space, allowing an intrathecal (i.t.) administration, is particularly interesting (Yaksh and Rudy, 1976). The pharmacological effects are then recorded, and two points can be underlined: appearance of these effects immediately after i.t. administration and effective intrathecal doses smaller than the systemic ones, suggesting a spinal origin, that is not a diffusion out of the spinal cord. A third interesting point, that is the comparison between "spinal" and "extra-spinal" effects can give further support to the hypothesis.

Another methodology to corroborate a spinal mechanism of action is to reduce competitively the effects of the drug injected via a systemic route (intravenous or i.v. for example) by an intrathecally administered antagonist. Furthermore, the magnitude of this decrease will give an estimation of the importance of the spinal mechanism with respect to the whole effect.

Finally, it is also possible to destroy the spinal biological system mediating the drug effect, inducing a reduction of the registered response after either systemic or intrathecal administration of the drug. For example, it has been recently shown (Tjolsen *et al.*,1991) that paracetamol elicits its analgetic effects partly by the spinal serotonergic system: these analgetic effects are reduced by an i.t. administration of 5-6 dihydroxytryptamine but not by 6-hydroxydopamine (neurotoxins specific of serotonergic and catecholaminergic neurons, respectively). It is also possible to induce an hypersensitive phenomenon by a complete spinal transection (Jensen *et al.*,1984).

Our aim was to apply this general methodology to the hypotensive effect of bromocriptine.

Hypotensive effects of intrathecally administered apomorphine.

The presence of a descending dopaminergic spinal system (DDSS) and of dopamine receptors in the spinal cord is now well demonstrated as are different pharmacological effects elicited by its stimulation (for a review see Feuerstein *et al.*,1988). We showed that i.t. administration of low doses of apomorphine, a mixed agonist for D-1 and D-2 receptors (Seeman, 1980) in conscious rats (Petitjean *et al.*,1984) elicited decreases in blood pressure and heart rate. These effects were dose-dependent and were competitively antagonized (rightward shift) by DA receptor antagonists. The decrease in blood pressure was mediated by stimulation of D-1 and D-2 dopamine receptors whereas the decrease in heart rate was only mediated by activation of spinal D-2 receptors (Pellissier and Demenge, 1991). Furthermore, these effects were not of the same magnitude according to the spinal rostrocaudal level of administration (maximum of both effects at upper thoracic level) and were in the line of a very restricted diffusion of tritiated apomorphine (Lahlou *et al.*,1990) along the spinal axis. The suggestion of a spinal mediation was corroborated by immediately appearing effects only at upper spinal levels whereas stereotyped movements, elicited by a diffusion out of the spinal cord, appeared only 2 to 3 min after i.t. administration of apomorphine at each studied spinal level, from mid-cervical to sacral localization. These results fulfilled the criteria defined above. Another preliminary finding supporting this hypothesis was the increase in hypotensive response induced by i.t. administered apomorphine caudally to a complete spinal transection.

So, as a dopamine receptor agonist was able to induce cardiovascular effects when injected directly in the spinal cord, it was possible to hypothesize that the hypotension induced by systemically administered bromocriptine could be mediated, at least in part, by spinal dopamine receptors, besides other sites of activity (Lokhandwala and Hedge, 1991).

Partial spinal mediation of hypotensive effects of intravenously administered bromocriptine.

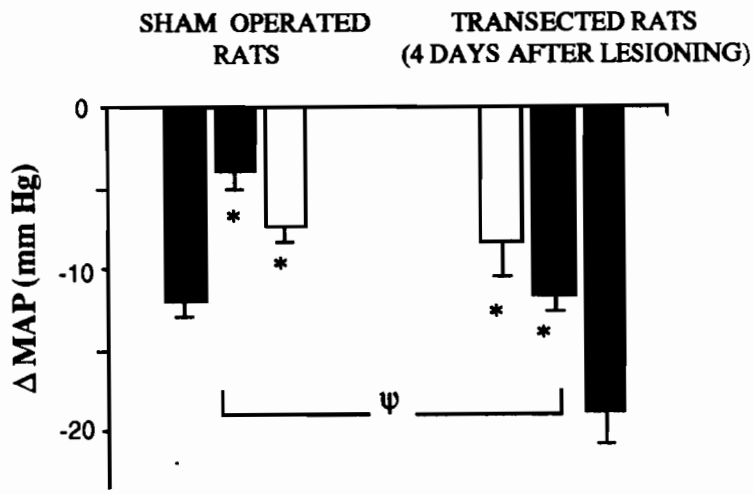
Bromocriptine, a dopamine D-2 receptor agonist, decreased dose -dependently arterial pressure in conscious rats, at bolus doses from 50 to 250 µg/ kg, administered by intravenous route and the duration of this hypotension was also dose-dependent. However, bromocriptine increased in the same time dose-dependently heart rate, and it was the reason why the experiments were performed on propranolol-treated rats, either conscious or anaesthetized with pentobarbital. The hypotension following an i.v. sub-maximal dose of bromocriptine was reduced by domperidone, a D-2 receptor antagonist, unable to cross blood-brain barrier (Laduron and Leysen, 1979), administered either by i.v. (spinal part not antagonized) or by i.t. route (peripheral part not antagonized). I.v. domperidone pretreatment reduced hypotension by about 65 % and i.t. domperidone pretreatment by about 35 % either in conscious or anaesthetized rats (Lahlou and Demenge, 1991).

Increased hypotensive responses after a complete spinal transection.

After a complete spinal mid-thoracic (at T5-T7 level) transection, an i.t. administration of 150 nmole of apomorphine induced the same hypotensive response as in sham-operated animals if performed rostrally to the lesion, that is at T2-T4 level but elicited an hypotension greater both in magnitude and in duration than in control rats if administered caudally, that is at T9-T10 level. This increase was corroborated by a leftward shift of the dose-hypotensive effect curve of i.t. apomorphine and was also shown with fenoldopam and quinpirole, selective D-1 and D-2 receptor agonists, respectively. Furthermore, this increased hypotensive effect was competitively antagonized by haloperidol, a specific dopamine receptor antagonist (Lahlou and Demenge, 1991). It could then be suggested that dopamine receptors located caudally to the transection were supersensitive or that this transection has destroyed a tonically inhibitor system acting upon DDSS. This latter hypothesis was supported by the lack of any activity of neuroleptics administered by i.t. route in normal rats (the degree of activity of an antagonist is closely related to the degree of activity of the system it is inhibiting). Whatever the mechanism

of this increased hypotensive response following i.t. apomorphine or dopamine receptor agonists after a complete spinal transection, it might be suggested that a similar lesion could also increase the hypotensive effect of systemically administered bromocriptine. This was shown in spinal concious rats, pretreated or not with propranolol, and treated with 150 µg/kg i.v. bromocriptine, which induced a greater and longer lasting hypotension, during the whole 8 day experimental period. This increase in hypotension was unchanged by i.v. domperidone , that is the peripheral part of the bromocriptine hypotensive effect was the same in spinal as in sham-operated rats (magnitude of the hypotension remaining after i.t. domperidone). In contrast, this increased hypotensive effect was antagonized by i.t. domperidone (Lahlou and Demenge,1991), suggesting this increase was fully related to spinal mechanisms (magnitude of hypotension remaining after i.v. domperidone greater in spinal rats than in control ones).

This finding may help to explain the increased orthostatic hypotension induced by D2 receptor agonists in Parkinsonian patients with spinal lesions.



Maximum decreases in mean arterial aortic pressure (ΔMAP mmHg) elicited by intravenous bromocryptine (150µg/kg) alone (■) or after i.v. (0,3mg/kg ; ⊠) or intrathecal (93nmol/rat ; at T9-T10 level ; □) domperidone in two conscious propanolol (0,5mg/kg i.v.) pretreated groups : sham operated rats and animals studied 4 days following spinal transection at T5-T7 level. Vertical bars indicate SEM (n=5-7 rats per group). In both normal and spinalized rats, i.t. and i.v. administered domperidone antogonized significantly (*: $p<0.05$; Mann-Whitney U test) the hypotension induced by i.v. domperidone was greater in spinal rats than in control ones (ψ : $p<0.05$ by aMann-Whitney U test)

Conclusion

It can be concluded that such a methodology, as described above, can help to corroborate a spinal mechanism of action for a drug, and may explain changes in effects according to spinal lesions.

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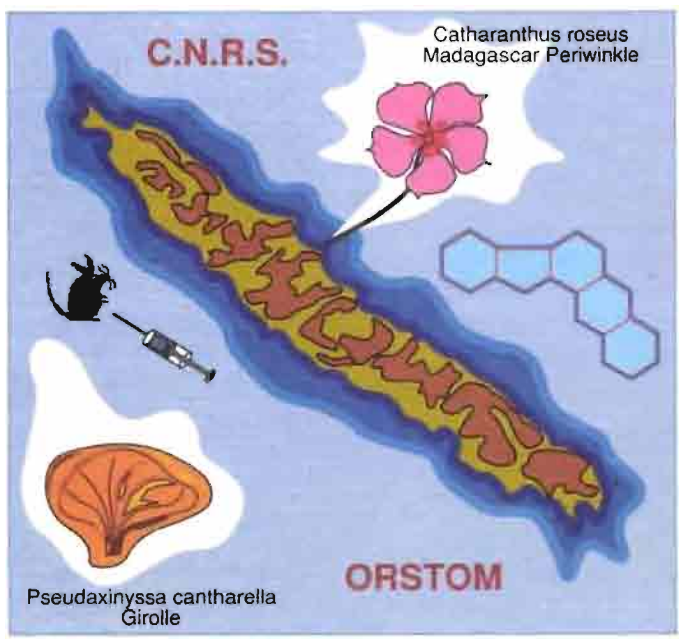

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