significance of the active metabolite norfluoxetine awaits further investigation.

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References

Benfield, P., Heel, R. C., Lewis, S. P. (1986) Fluoxetine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in depressive illness. Drugs 32: 481–508

Bergstrom, R. F., Lemberger, L., Farid, N. A., Wolen, R. L. (1988) Clinical pharmacology and pharmacokinetics of fluoxetine: a review. Br. J. Psychiatry 153 (Suppl. 3): 47–50

Caccia, S., Bizzi, A., Coltro, G., Fracasso, C., Frittoli, E., Mennini, T., Garattini, S. (1992) Anorectic activity of fluoxetine and norfluoxetine in rats: relationship between brain concentrations and in-vitro potencies on monoaminergic mechanisms. J. Pharm. Pharmacol. 44: 250–254

Caccia, S., Cappi, M., Fracasso, C., Garattini, S. (1990) Influence of dose and route of administration on the kinetics of fluoxetine and its metabolite norfluoxetine in the rat. Psychopharmacology 100: 509–514

De Lean, A., Munson, P. J., Rodbard, D. (1978) Simultaneous analysis of families sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves. Am. J. Physiol. 235: E97–E102

Ferguson, J. M., Feighner, J. P. (1987) Fluoxetine-induced weight loss in overweight non-depressed humans. Int. J. Obes. 11 (Suppl. 3): 163–170

Freeman, C. P. L. (1988) Drug treatment for bulimia and bulimia nervosa. Psychopharmacology 96 (Suppl.): 124

Garattini, S., Caccia, S., Mennini, T., Samanin, R. (1991) Progress report on drugs inducing anorexia by affecting brain serotonin. In: Rothwell, N. J., Stock, M. J. (eds) Obesity and Cachexia. Wiley, New York, pp 227–240

Gobbi, M., Frittoli, E., Mennini, T., Garattini, S. (1992) Releasing activities of d-fenfluramine and fluoxetine on rat hippocampal synaptosomes preloaded with ^{3}H-serotonin. Naunyn Schmiedebergs Arch. Pharmacol. 345: 1–6

Goudie, A. J., Thornton, E. W., Wheeler, T. J. (1976) Effects of Lilly 110140, a specific inhibitor of 5-hydroxytryptamine uptake, on food intake and on 5-hydroxytryptophan-induced anorexia. Evidence for serotoninergic inhibition of feeding. J. Pharm. Pharmacol. 28: 318–320

Nathan, C., Rolland, Y. (1987) Pharmacological treatments that affect CNS activity: serotonin. Ann. NY Acad. Sci. 499: 277–296

Parli, C. J., Hicks, J. (1974) In vivo demethylation of Lilly 110140: 3(p-TRI-fluoromethylphenoxy)-*N*-methyl-3-phenylpropylamine to an active metabolite-Lilly 103947. Fed. Proc. 33: 560

Samanin, R., Garattini, S. (1990) The pharmacology of serotoninergic drugs affecting appetite. In: Wurtman, R. J., Wurtman, J. J. (eds) Nutrition and the Brain. Vol. 8, Raven Press, New York, pp 163–192

Wong, D. T., Fuller, R. W. (1987) Serotonergic mechanisms in feeding. Int. J. Obes. (Suppl. 2): 125–133

Wong, D. T., Reid, L. R., Threlkeld, P. G. (1988) Suppression of food intake in rats by fluoxetine: comparison of enantiomers and effects of serotonin antagonists. Pharmacol. Biochem. Behav. 31: 475–479

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Further studies on the anti-nociceptive effect of vasopressin

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Abstract—The possible interactions of pathways which mediate anti-nociception when stimulated by α_2-adrenoceptor agonists and arginine vasopressin (AVP) were investigated. Yohimbine, an α_2-antagonist, failed to modify the anti-nociceptive response of AVP. However, clonidine pretreatment, in sub-effective and effective doses, potentiated the anti-nociceptive response of a sub-effective dose of AVP. This potentiation was attenuated by yohimbine and completely anatagonized by naloxone. These studies suggest that pathways related to the opioidergic system and those stimulated by α_2-agonists may be utilized by AVP in eliciting the anti-nociceptive response.

Attempts have been made in recent years to understand the mechanism responsible for the anti-nociceptive activity produced by arginine vasopressin (AVP) recorded in different animal models. This effect has been reported to be independent of the opioidergic system (Berkowitz & Sherman 1982; Hart & Oluyomi 1990). An important non-opiate pathway for eliciting anti-nociception is one which is stimulated by α_2-adrenoceptor agonists. α_2-Agonists, such as clonidine produced a significant anti-nociceptive response which was antagonized by yohimbine and unaffected by naloxone (Paalzow & Paalzow 1976; Spaulding et al 1979; Chance 1983).

Even though opioidergic and α_2-adrenergic anti-nociception are independent, an interaction between them and a common pathway have been proposed (Spaulding et al 1979; Ramaswamy et al 1981, 1983b). The possible interaction between AVP and α_2-agonists in eliciting anti-nociception has not been examined. In the present study, an effort has been made to analyse such an inter-play.

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Materials and methods

Male Swiss albino mice, 25–30 g, housed in a 12 h dark: 12 h light cycle with free access to food and water were used. Anti-nociception was tested using the acetic acid-induced abdominal constriction assay. The onset and number of abdominal constrictions were noted for a period of 10 min following the injection of acetic acid (0·6%; 10 mL kg^{-1}; i.p.). The anti-nociceptive effect of clonidine (0·1 and 1·0 μg kg^{-1}; i.p.) and AVP (0·8 and 4·0 μg kg^{-1}; i.v.) were recorded by administering them 15 or 10 min, respectively, before acetic acid challenge.

Table 1. The influence of AVP, clonidine, yohimbine and naloxone alone and in combination on acetic acid-induced abdominal constriction in mice.

First treatment (mg kg^{-1})	Second treatment (μg kg^{-1})	Third treatment (μg kg^{-1})	Abdominal constrictions		
			Onset (s)	Number	Inhibition (%)
Saline	Saline	Saline	209·2 ± 18·3	22·8 + 1·7	—
Saline	Clonidine (0·1)	Saline	242·0 ± 17·4	19·3 ± 1·4	15·4
Saline	Clonidine (1·0)	Saline	306·6 ± 41·3[a]	13·0 ± 1·5[a]	43·0
Saline	Saline	AVP (0·8)	199·0 ± 13·5	19·6 ± 0·7	14·1
Saline	Saline	AVP (4·0)	313·0 ± 14·7[a]	10·6 ± 0·8[a]	53·6
Saline	Clonidine (0·1)	AVP (0·8)	331·0 ± 40·2[a,b]	11·8 ± 1·6[a,b]	48·3
Saline	Clonidine (1·0)	AVP (0·8)	434·0 ± 45·5[a,b]	3·6 ± 0·9[a,b]	84·3
Yohimbine (1·0)	Saline	Saline	220·4 ± 18·7	20·8 ± 0·9	8·8
Yohimbine (1·0)	Clonidine (1·0)	Saline	185·0 ± 2·9[c]	21·3 ± 1·8[c]	6·6
Yohimbine (1·0)	Saline	AVP (4·0)	330·0 ± 36·7[a]	8·0 ± 1·7[a]	65·0
Yohimbine (1·0)	Clonidine (1·0)	AVP (0·8)	354·0 ± 30·1[a,d]	8·1 ± 1·2[a,d]	64·5
Naloxone (1·0)	Saline	Saline	205·0 ± 12·0	24·6 ± 1·2	—
Naloxone (1·0)	Clonidine (1·0)	AVP (0·8)	276·9 ± 18·9[e]	16·4 ± 1·42[e]	28·1

The values are mean ± s.e.m. of six experiments. [a] $P < 0·01$ compared with saline–saline–saline value. [b] $P < 0·05$ compared with saline–saline–AVP (0·8) and saline–saline–clonidine (0·1) values. [c] $P < 0·05$ compared with saline–clonidine (1·0)–saline value. [d] $P < 0·05$ and [e] $P < 0·01$ compared with saline–clonidine (1·0)–AVP (0·8) value.

The influence of yohimbine (1 mg kg^{-1}; i.p.) on clonidine (1 μg kg^{-1}; i.p.)- and AVP (4 μg kg^{-1}; i.v.)-induced anti-nociception was examined by injecting yohimbine 15 min before clonidine or AVP. In separate groups of animals, the influence of clonidine (0·1 or 1·0 μg kg^{-1}) and AVP (0·8 μg kg^{-1}; i.v.) on the abdominal constrictions was studied by administering clonidine 15 min before AVP.

The role of α_2-adrenergic mechanisms and the opioidergic system on the changes in the AVP anti-nociception produced by clonidine was examined by exposing the animals either to yohimbine (1 mg kg^{-1}; i.p.) or to naloxone (1 mg kg^{-1}; i.p.) 15 min before clonidine. The changes induced by yohimbine (1 mg) and naloxone (1 mg) on the abdominal constrictions were recorded independently. Saline-treated animals served as controls.

The results were statistically analysed initially by analysis of variance followed by Dunnett's t-test.

Results

The data are summarized in Table 1. Clonidine (0·1 μg kg^{-1}) did not significantly modify the abdominal constrictions but a dose of 1.0 μg kg^{-1} significantly delayed the onset and decreased the number of abdominal constrictions. AVP at a dose of 0·8 μg did not significantly modify either the onset or the number of abdominal constrictions whereas 4·0 μg of AVP significantly delayed the onset and decreased the number of abdominal constrictions. Exposure to sub-effective doses of clonidine (0·1 μg) and AVP (0·8 μg) resulted in a significant delay in the onset and reduction in the abdominal constrictions. Further, a similar but enhanced response was recorded in animals treated with clonidine (1·0 μg) and AVP (0·8 μg) at 15 min intervals.

This enhancement was comparatively less in yohimbine-pretreated animals and was almost absent in naloxone-pretreated animals. Yohimbine pretreatment significantly antagonized the effect of clonidine while it did not affect that of AVP (4·0 μg kg^{-1}). The antagonists themselves in the doses employed did not modify significantly the abdominal constrictions.

Discussion

In agreement with the previous reports (Berkowitz & Sherman 1982; Hart & Oluyomi 1990), AVP when administered i.v. produced a significant anti-nociception as tested in the acetic acid-induced abdominal constriction assay. The α_2-adrenergic pathways appear not to contribute to the AVP-induced anti-nociceptive response as the AVP response was insensitive to yohimbine. It is clear that both clonidine and AVP act through non-opiate mechanisms to elicit anti-nociception (Spaulding et al 1979; Berkowitz & Sherman 1982). However, clonidine-induced anti-nociception was sensitive to yohimbine (Paalzow & Paalzow 1976) while that of AVP was not sensitive as shown in this study. Hence, it may be stated that AVP and clonidine might utilize different pathways in this action.

The results of the experiments involving clonidine and AVP treatment, however, showed that a combination of sub-effective doses of clonidine and AVP produced a significant anti-nociceptive response. Furthermore, clonidine pretreatment potentiated the anti-nociceptive effect of a sub-effective dose of AVP. These findings suggest some role for α_2-adrenergic pathways in the anti-nociceptive response of AVP. This suggestion was supported by the observation that this potentiation was reduced (84·3–64·5%) in yohimbine-pretreated animals.

It has been previously reported that clonidine and morphine, though acting through different mechanisms, potentiate the anti-nociceptive response of each other (Spaulding et al 1979). Therefore, it was suggested that these agents, though acting independently, might utilize a common pathway in their anti-nociceptive responses. Similarly, AVP which was reported to act through a non-opiate mechanism (Berkowitz & Sherman 1982) has been shown to potentiate the anti-nociceptive effect of prolactin (Ramaswamy et al 1991) which essentially utilizes opioid pathways (Ramaswamy et al 1983a). This potentiation was naloxone-sensitive which suggested that it was mediated through an opioidergic system (Ramaswamy et al 1991). In the present study, the possible involvement of opiate pathways in the potentiation recorded for AVP-induced anti-nociception by clonidine was tested by employing naloxone. The reduction of this potentiation (84·3–28·1%) in naloxone-pretreated animals favours a role for an opiate mechanism in this action. This finding further supports the contention that α_2-adrenergic and opiate mechanisms utilize related pathways and also suggest that AVP could act through more than one pathway to elicit an anti-nociceptive response.

References

Berkowitz, B. A., Sherman, S. (1982) Characterisation of vasopressin analgesia. J. Pharmacol. Exp. Ther. 220: 329–334

Chance, W. T. (1983) Clonidine analgesia: tolerance and cross tolerance to autoanalgesia. Life Sci. 33: 2241–2246

Hart, S. L., Oluyomi, A. O. (1990) Vasopressin and stress-induced anti-nociception in the mouse. Br. J. Pharmacol. 99: 243–246

Paalzow, G., Paalzow, L. (1976) Clonidine anti-nociceptive activity: effects of drugs influencing central monoaminergic and cholinergic mechanisms in the rat. Naunyn- Schmiedebergs Arch. Pharmacol. 292: 119–126

Ramaswamy, S., Padmanabha Pillai, N., Bapna, J. S. (1983a) Analgesic effect of prolactin: possible mechanism of action. Eur. J. Pharmacol. 96: 171–173

Ramaswamy, S., Padmanabha Pillai, N., Gopalakrishnan, V., Ghosh, M.N. (1981) Influence of clonidine on the acute tolerance pattern to morphine induced analgesia and sensitivity changes in mice. Life Sci 28: 2237–2241

Ramaswamy, S., Padmanabha Pillai, N., Gopalakrishnan, V., Ghosh, M.N. (1983b) Effect of clonidine on the chronic morphine tolerance and on the sensitivity of the smooth muscles in mice. Ibid. 33: 1167–1172

Ramaswamy, S., Shewade, D.G., Tyagi, M.G., Bapna, J.S. (1991) Influence of arginine vasopressin on prolactin induced analgesia in mice. Medical Science Research. 19: 871–872

Spaulding, T.C., Fielding, S., Venofro, J.J., Lal, H. (1979) Antinociceptive activity of clonidine and its potentiation of morphine analgesia. Eur. J. Pharmacol. 58: 19–25

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Effects of benzodiazepine administration on A_1 adenosine receptor binding in-vivo and ex-vivo

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Abstract—The adenosine receptor has been implicated in the central mechanism of action of benzodiazepines. The specific binding of an A_1-selective adenosine antagonist radioligand, [^{3}H]8-cyclopentyl-1,3-dipropylxanthine, was measured in-vivo in mice treated with alprazolam (2 mg kg^{-1}, i.p.), lorazepam (2 mg kg^{-1}, i.p.) and vehicle. Binding studies were performed in-vivo and ex-vivo in mice receiving continuous infusion of alprazolam (2 mg kg^{-1} day^{-1}), lorazepam (2 mg kg^{-1} day^{-1}) and vehicle by mini-osmotic pumps for 6 days. Continuous infusion of alprazolam and lorazepam significantly decreased specific binding by 34 and 53%, respectively, compared with vehicle treatment ($P < 0.01$). Single doses of alprazolam and lorazepam induced a similar trend in specific binding in-vivo ($P = 0.07$). There were no alterations in A_1-receptor density (B_{max}) or affinity (K_d) in cortex, hippocampus or brainstem in ex-vivo studies. Benzodiazepine treatment may diminish A_1- receptor binding in-vivo by inhibiting adenosine uptake or by direct occupancy of the A_1 adenosine receptor recognition site.

The central effects of benzodiazepines are mediated by molecular mechanisms which have not been completely elucidated. Benzodiazepines are known to bind to a high-affinity benzodiazepine receptor site on a supramolecular $GABA_A$ complex, enhancing binding of γ-aminobutyric acid (GABA) to its recognition site and altering coupling to its effector. However, benzodiazepine-induced enhancement of GABA-ergic synaptic transmission or its antagonism do not explain all of the clinical effects of benzodiazepines. Other mechanisms of action have been considered including the interaction of benzodiazepines with the neuromodulator adenosine. Adenosine is an endogenous neuromodulator which binds to its own recognition sites, A_1 and A_2, and like benzodiazepines, adenosine agonists are known to be sedating, motor-depressant and anticonvulsant (Phillis & O'Regan 1988).

Benzodiazepines reverse the behavioural effects of adenosine receptor antagonists, such as caffeine. Diazepam reverses caffeine-induced restlessness, tension, alertness and arousal in man (Roache & Griffiths 1987). In animal models, interactions between benzodiazepine and adenosine agonist and antagonist agents on locomotor activity, suppressed behaviours and conflict behaviours have been demonstrated (Crawley et al 1981; Polc et al 1981; Barraco et al 1984; Kaplan et al 1990). However, a lack of interaction between diazepam and adenosine agonists was shown in another behavioural study (Commissaris et al 1990). Consistent interactions have been seen with convulsant effects, since adenosine agonists suppress the induction of seizures by bicuculline, a GABA-ergic proconvulsant agent (Franklin et al 1989), and benzodiazepines inhibit caffeine-induced seizures (Marangos et al 1981; Chweh et al 1986).

Functional responses of the adenosine receptor system have been examined by measuring adenosine-induced depression of evoked activity in neurons. Alterations of adenosine-evoked depression by benzodiazepine have been consistently found, suggesting their role in modulating adenosinergic responses (Phillis 1979; Mally et al 1990).

Various alterations of the specific components of the adenosine receptor system by benzodiazepines have shown that benzodiazepines inhibit the uptake and release of adenosine at a specific high-affinity site, resulting in increased concentrations of extracellular adenosine (Phillis et al 1980a, b; Bender et al 1980). Adenosine-uptake-inhibiting agents and benzodiazepines similarly block adenosine uptake (Bender & Hertz 1986). A correlation between inhibition of benzodiazepine binding and inhibition of adenosine uptake has been found with various drugs (Wu et al 1980). Additionally, a correlation between adenosine-uptake inhibition and inhibition of seizure-induction by the GABA-ergic agent, pentetrazol, has been described (Chweh et al 1984). These correlations suggest relationships between adenosine-uptake-inhibition and benzodiazepine receptor binding and function.

Adenosine and diazepam are structurally similar with a flat, fused heterocyclic ring structure and neutral charge, suggesting that cross-reactivity of drugs could occur between the two receptor systems. Benzodiazepines bind weakly to adenosine receptors in-vitro at micromolar concentrations while binding to

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