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Modulating effect of the nootropic drug, piracetam on stress- and subsequent morphine-induced prolactin secretion in male rats

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1 The effect of the nootropic drug, piracetam on stress- and subsequent morphine-induced prolactin (PRL) secretion was investigated *in vivo* in male rats, by use of a stress-free blood sampling and drug administration method by means of a permanent indwelling catheter in the right jugular vein.

2 Four doses of piracetam were tested (20, 100, 200 and 400 mg kg^{-1}), being given intraperitoneally 1 h before blood sampling; control rats received saline instead. After a first blood sample, rats were subjected to immobilization stress and received morphine, 6 mg kg^{-1}, 90 min later.

3 Piracetam had no effect on basal plasma PRL concentration.

4 While in the non-piracetam-treated rats, stress produced a significant rise in plasma PRL concentration, in the piracetam-pretreated rats PRL peaks were attenuated, especially in the group given 100 mg kg^{-1} piracetam, where plasma PRL concentration was not significantly different from basal values. The dose-response relationship showed a U-shaped curve; the smallest dose had a minor inhibitory effect and the highest dose had no further effect on the PRL rise.

5 In unrestrained rats, morphine led to a significant elevation of plasma PRL concentration. After the application of immobilization stress it lost its ability to raise plasma PRL concentration in the control rats, but not in the piracetam-treated rats. This tolerance was overcome by piracetam in a significant manner but with a reversed dose-response curve; i.e. the smaller the dose of piracetam, the higher the subsequent morphine-induced PRL peak.

6 There is no simple explanation for the mechanism by which piracetam induces these contradictory effects. Interference with the excitatory amino acid system, which is also involved in opiate action, is proposed speculatively as a possible mediator of the effects of piracetam.

Keywords: Piracetam; nootropic drug; morphine; opioid; opiate; tolerance; immobilisation; stress; prolactin

Introduction

Piracetam is the prototype of the nootropic drugs, which are claimed to improve the higher telencephalic activities in the brain and to restore deficient higher nervous activity without affecting subcortical functions (Giurgea, 1982). Unlike other psychotropic drugs, nootropics do not cause sedation or stimulation and are completely devoid of toxic effects, even when administered in very high doses. Nootropics were found to have beneficial effects on learning and memory, especially in the case of cerebral impairment (Banfi & Dorigotti, 1986). Animal experiments as well as clinical trials in human subjects have shown that nootropics display a protective or restoring effect against various types of brain insults such as hypoxia (Nikolova *et al.*, 1984), trauma (Richardson, 1977) and drug intoxication (Moyersoons & Giurgea, 1974; for review see Vernon & Sorkin, 1991). Especially in the case of so-called depressant drugs like alcohol (Dencker & Wilhelmson, 1978), barbiturates (Moyersoons & Giurgea, 1974) or heroin (Chaudhry *et al.*, 1990), piracetam attenuates toxic effects or favours less severe withdrawal symptoms.

Up until now, the mechanism of action of these nootropic drugs has not been elucidated. Though piracetam is a cyclic derivative of γ-aminobutyric-acid (GABA), it does not display any GABA-ergic property or affinity. Moreover, piracetam and its congeners (e.g. oxiracetam, aniracetam, pramiracetam,...) do not possess a significant affinity for any known receptor binding site (for review see Gouliaev & Senning, 1994). It has been shown that nootropics can potentiate excitatory neurotransmission (Satoh *et al.*, 1986; Marchi *et al.*, 1990; Pugliese *et al.*, 1990) and even slow desensitization of the excitatory receptors involved (Tang *et al.*, 1991).

Opiates, like morphine, and opioid peptides stimulate prolactin (PRL) secretion. Some types of stress also cause a naloxone-reversible rise in PRL release, suggesting action through an opioid system (Bruni *et al.*, 1977; Van Vugt *et al.*, 1978; Siegel *et al.*, 1982; Samson *et al.*, 1985). Indeed, the release of endogenous opioids, in particular β-endorphin, may play a major role in the initiation of stress-induced PRL secretion (Van Vugt *et al.*, 1978; Ragavan & Frantz, 1981).

Because of the central effects of piracetam and its beneficial influence on the effects of depressant or addictive drugs, this study was set up to investigate in rats the influence of piracetam on opioid-mediated stress-induced PRL secretion as well as on the tolerance to morphine-induced PRL secretion occurring after stress exposure.

Methods

Animals

Experiments were performed on adult male Wistar rats weighing 250 to 300 g (KUL, Leuven, Belgium). The animals were housed individually in wire-bottomed cages and food and water were freely available. Lights were on from 07 h 00 min to 19h 00min and the room temperature was constant at 22°C. All blood sampling was performed between 13 h 00 min and 16 h 00 min.

Surgery

For i.v. drug administration and serial blood sampling, a permanent silicone elastomer catheter was implanted in the right jugular vein under sodium pentobarbitone (60 mg kg^{-1} intraperitoneally) anaesthesia, as described previously by Harms & Ojeda (1974).

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Pharmacological agents

Piracetam was kindly donated by UCB (Braine-L'Alleud, Belgium). Morphine-HCl and naloxone-HCl were purchased from Belgopia (Louvain-La-Neuve, Belgium). All solutions were made in physiological saline (0.9% NaCl solution).

Experimental procedures

All experiments were conducted at least 4 days after surgery. On the morning of the experiment a PE60 tubing (Dow Corning, Midland, MI, U.S.A.) was affixed to the free end of the jugular catheter and extended from the animal to the outside of the cage, so that the rat could move freely and was not aware of blood sampling or drug administration, thus avoiding uncontrolled stress. The rats were left undisturbed for about 3 h before the first blood sampling.

Blood samples (0.9 ml) were collected into heparinized tubes by means of a peristaltic pump (Gilson, Villiers-le-Bel, France). The samples were immediately centrifuged and plasma was stored at −20°C until assayed for PRL. Red blood cells were resuspended in saline and reinfused after the following blood sample to minimize the effect of blood loss.

For the experiments with unrestrained rats, morphine 6 mg kg^{-1} was administered through the catheter after a first basal blood sampling. For the immobilization stress experiments, each rat was placed in a narrow cylindrical glass cage allowing almost no freedom of movement. The stress was applied after a first basal blood sampling till the end of the experiment. For the naloxone experiments, naloxone 3 mg kg^{-1} was administered i.v. through the catheter, after a first blood sampling and just before the stress application.

For the piracetam experiments, all animals received an i.p. injection of piracetam (20, 100, 200 or 400 mg kg^{-1} body weight) 1 h before the first (basal) blood sample. Control rats received an i.p. injection of saline instead of piracetam. Morphine was administered i.v. through the catheter, 90 min after the beginning of the stress application.

Hormone assay and data analysis

Plasma samples were assayed for PRL in duplicate by double-antibody radioimmunoassay. Prolactin for iodination (NIADDK-rPRL 1-6) and standard (NIADDK-rPRL-RP3) were kindly supplied by the NIADDK (Torrance, CA, U.S.A.) and the National Hormone and Pituitary Program (Baltimore, MD, U.S.A.). The first antibody (rabbit polyclonal rPRL antibody 6-10/90) was used at a final dilution of 1/80,000 and had higher specificity in terms of its low cross-reactivity with anterior pituitary hormones other than PRL, compared to the NIADDK antiserum anti-rPRL-S9. The assays were run according to the NIADDK protocol. The optimal detectability in 100 µl undiluted plasma ranged from 0.25 to 50 ng ml^{-1}. Samples exceeding this upper limit were diluted in assay buffer. Intra- and interassay coefficients of variation were less than 6% and 8% respectively.

Data were analyzed by means of the Exstatix 1.01 programme (Select Micro Systems Inc., Yorktown Heights, NY, U.S.A.) on an Apple MacIntosh computer. ANOVA (Analysis of variance) with an *a posteriori* Scheffé test was used for analysis of the results. A probability level of $P<0.05$ was considered significant. Mean and s.e.mean values were calculated separately, resulting in n−1 degrees of freedom for s.e.mean.

Results

Effect of morphine or immobilization stress on PRL secretion (Table 1)

All rats showed a significant rise in plasma PRL concentration after administration of morphine (6 mg kg^{-1}). Rats subjected to immobilization stress also had a significantly elevated plasma PRL concentration. Though immobilization was maintained until after the last blood sampling (45 min after onset of stress), plasma PRL concentration returned to basal values after 30 min. The rise of plasma PRL concentration by immobilization stress was completely inhibited by prior administration of an opiate antagonist, namely naloxone 3 mg kg^{-1}.

Effect of piracetam pretreatment on stress-induced PRL secretion (Figure 1)

Control rats that did not receive piracetam pretreatment responded to stress with a significant rise in plasma PRL concentration, but in rats pretreated with 100 and 200 mg kg^{-1} piracetam, the stress-induced PRL peaks were significantly attenuated ($P<0.05$). The inhibition of the PRL rise was the greatest after 100 mg kg^{-1} piracetam, since in this group the plasma PRL concentration was no longer significantly different from basal PRL concentration. Rats pretreated with the lowest dose, namely 20 mg kg^{-1} piracetam, also had significantly attenuated PRL peaks, although this attenuation was much smaller. The higher dose of piracetam (400 mg kg^{-1}) had no significant effect on stress-induced PRL release. Consequently, the effect of piracetam on stress-induced PRL secretion showed a U-shaped dose-response curve.

Effect of piracetam on stress-induced tolerance to morphine (Figure 1)

Morphine administered after stress application lost its ability to stimulate PRL release. Thus, control rats treated with morphine (6 mg kg^{-1}) 90 min after the onset of stress showed no increase in plasma PRL concentration, suggesting that rats subjected to stress were 'tolerant' to morphine. However, no such tolerance was evident in piracetam-treated rats. Hence, all piracetam-pretreated rats showed a strong significant rise in plasma PRL concentration 15 min after morphine adminis-

Table 1 Plasma prolactin concentration in rats after morphine, immobilization stress and naloxone prior to the immobilization stress respectively, at time = 15 min

	Plasma prolactin (ng ml^{-1})			
Time (min)	0	15	30	45
Morphine 6 mg kg^{-1} ($n=12$)	2.80 ± 0.49	27.16 ± 4.44*	10.00 ± 2.09*	1.63 ± 0.13
Immobilization ($n=10$)	2.31 ± 0.37	28.69 ± 8.82*	15.86 ± 4.07*	2.47 ± 0.19
Naloxone 3 mg kg^{-1} and immobilization ($n=9$)	2.84 ± 0.80	2.75 ± 0.68	2.69 ± 0.69	2.64 ± 0.54

Values are means ± s.e.mean.
*$P<0.05$ compared to basal value at time = 0 min; (ANOVA + Scheffé test).

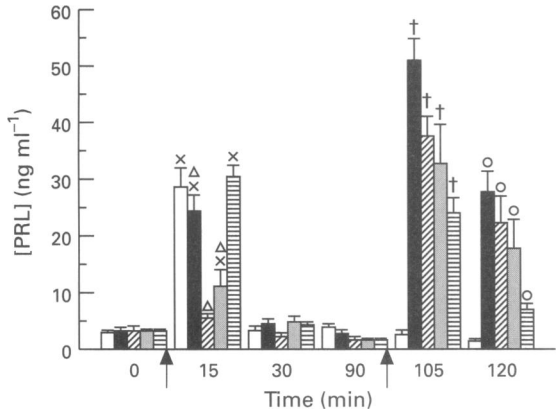

Figure 1 Plasma concentrations of prolactin (means ± s.e.mean) in rats pretreated with saline (open columns, $n=15$) or piracetam 20 mg kg^{-1} (solid columns, $n=15$), 100 mg kg^{-1} (diagonally striped columns, $n=16$), 200 mg kg^{-1} (stippled columns, $n=16$) or 400 mg kg^{-1} (horizontally striped columns, $n=17$) i.p. 1 h before the first blood sampling. Immobilization stress (first arrow) was applied after the first basal blood sample (time = 0). Rats received 6 mg kg^{-1} of morphine (second arrow) after blood sampling at time = 90 min. $^{\triangle}P<0.05$ compared to controls at time = 15 min (ANOVA + Scheffé test). $^{\times}P<0.05$ compared to basal values at time = 0 min (ANOVA + Scheffé test). $^{\dagger}P<0.05$ compared to controls at time = 105 min (ANOVA + Scheffé). $^{\circ}P<0.05$ compared to controls at time = 120 min (ANOVA + Scheffé test)

tration. The higher the dose of piracetam, the smaller the morphine-induced rise in PRL secretion, showing a tendency towards a reversed dose-response curve. All differences between groups were significant, except the difference between the 100 and 200 mg kg^{-1} piracetam group. The same pattern, with the same statistical significance, was observed at 120 min.

When the morphine-induced peaks in plasma PRL concentration in unrestrained rats were compared with those of the restrained rats pretreated with piracetam, the peak PRL concentration after morphine administration (see Figure 1 at 105 min) was significantly higher in the rats pretreated with 20 and 100 mg kg^{-1} piracetam; the peaks in rats that had been given 200 and 400 mg kg^{-1} piracetam (see Figure 1 at 105 min) showed significant differences between each other but were not significantly different from the morphine-induced PRL peak in unrestrained rats without piracetam pretreatment (see Table 1 at time = 15 min).

Discussion

From our results it is clear that piracetam has no effect on basal PRL secretion. This contrasts with a study by Nybäck and coworkers (1979), who claimed that piracetam can stimulate PRL secretion. However, the dose used in this study (5 g kg^{-1}) was extremely high and, as the authors mentioned themselves but did not include in their results, lower doses had no effect. Moreover, it should be noted that their basal PRL values were about 10 times higher than ours, probably due to their method of blood collection (the rats were decapitated and blood was collected from the carcasses), which makes it difficult to compare the results.

It is not unusual for nootropics to be without effect in basal or 'normal' conditions. Several studies conclude that nootropics probably do not have any activity on their own, but require a stimulus or 'marginal situation' in order to exert their effects, e.g. potentiation or modulation of an already present activity (Marchi *et al.*, 1990; Nickolson & Wolthuis, 1976; Gouliaev & Senning, 1994). This is the case in the present study, since piracetam inhibited stress-induced PRL secretion but did not affect basal PRL values. Depending on the dose of piracetam, the inhibition was stronger or weaker, with a U-shaped response curve (see Figure 1).

Prolonged consumption of piracetam has been shown to decrease plasma β-endorphin concentrations 3 fold (Vakulina *et al.*, 1990) in rats. Since β-endorphin is involved in stress-induced PRL secretion (Van Vugt *et al.*, 1978; Ragavan & Frantz, 1981), these effects of piracetam on β-endorphin are in agreement with our findings showing an inhibition of the stress-induced effects. In the same study, Vakulina and coworkers showed that piracetam increased the cyclic AMP content in the rat brain cortex; by contrast, the bulk of evidence suggests that acute opioid receptor stimulation leads to a decrease in cyclic AMP concentration, because of a G-protein-mediated inhibition of adenylate cyclase (Childers *et al.*, 1991; Nestler, 1992). Two other studies also attributed 'anti-opiate' effects to piracetam. Piracetam blocked the analgesic effect of fentanyl (also an opioid μ-agonist) in rats (Krylova *et al.*, 1988) and antagonized the effects of morphine on respiration and pain perception (Chichenkov *et al.*, 1990) without interaction with the opioid receptors. Moreover, piracetam was also able to prevent morphine-induced corticosterone secretion in rats (Kóranyi & Endröczi, 1983).

The U-shaped dose-action curve of piracetam found in the present study is common among nootropics. In the literature, a considerable number of studies mention the fact that piracetam in lower doses exerts a significant effect while in higher doses the effect may either dissipate until it completely disappears (Satoh *et al.*, 1986; Marchi *et al.*, 1990; Pugliese *et al.*, 1990; Pizzi *et al.*, 1993) or reverse direction (Raiteri *et al.*, 1992; Spignoli *et al.*, 1986), resulting in a reversed dose-response curve or a U- or bell-shaped curve respectively.

While piracetam inhibits stress-induced PRL secretion, it appears to be stimulatory on the succeeding morphine administration. In control rats, exposure to stress effectively abrogated the increase in PRL secretion evoked by a subsequent injection of morphine (see Table 1). In a previous study we demonstrated that the failure to respond to morphine is not due to depletion of PRL stores, but resulted from a stress-induced tolerance of μ-opioid receptor mediated events on which morphine also acts (Matton *et al.*, 1991). In the piracetam-pretreated rats, no such tolerance to morphine was apparent (see Figure 1), confirming that depletion of PRL could not be responsible for the lack of response. The effects of piracetam were inversely related to the dose, i.e. the lower the dose of piracetam, the higher the PRL peak after morphine administration.

So far we have no explanation for the mechanism by which piracetam can influence opioid action. It is clear that it does not interact with any of the opioid receptors (Bering & Müller, 1985; Gouliaev & Senning, 1994). Thus other receptors or neurotransmitter systems might be involved. A possible level of interaction for piracetam on opioid-induced PRL secretion might be upon dopaminergic neurotransmission, since dopamine is the main regulatory factor in PRL secretion (MacLeod *et al.*, 1988) and piracetam has been shown to influence the turnover of dopamine in the striatum (Rägo *et al.*, 1981). However, this fails to account for the observed effects, since basal plasma PRL concentration should also be affected in this case.

Another possible explanation for our findings might be sought at the level of excitatory amino acid (EAA) neurotransmission. There is now firm evidence that nootropics modulate EAA mechanisms. Glutamate is the main neurotransmitter of excitatory cell signal processes and its release after cell depolarization has been shown to be potentiated by nootropics (Marchi *et al.*, 1990). Moreover, nootropics enhance neurotransmission of the different types of glutamate receptors (Pugliese *et al.*, 1990; Tang *et al.*, 1991; Copani *et al.*, 1992; Tsuzuki *et al.*, 1992). Since several recent studies suggest that the EAA system is also involved in opioid action, both after acute immobilization stress (Tocco *et al.*, 1991) and in

tolerance and withdrawal conditions (Akaoka & Aston-Jones, 1991; Aghajanian *et al.*, 1994), and since NMDA-antagonists can attenuate morphine-induced tolerance (Marek *et al.*, 1991; Trujillo & Akil, 1991; Tiseo & Inturrisi, 1993), it is possible that it is through this EAA system that piracetam exerts its effects on opioid stress and tolerance. However, no complete explanation can so far be provided and possibly even more complex interactions with neurotransmitters, second messengers or ion channels might be involved.

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References

AGHAJANIAN, G.K., KOGAN, J.H. & MOGHADDAM, B. (1994). Opiate withdrawal increases glutamate and aspartate efflux in the locus coeruleus: an in vivo microdialysis study. *Brain Res.*, **636,** 126–130.

AKAOKA, H. & ASTON-JONES, G. (1991). Opiate withdrawal-induced hyperactivity of locus coeruleus neurons is substantially mediated by augmented excitatory amino acid input. *J. Neurosci.*, **11,** 3830–3839.

BANFI, S. & DORIGOTTI, L. (1986). Experimental behavioural studies with oxiracetam on different types of chronic cerebral impairment. *Clin. Neuropharmacol.*, **9,** 19–26.

BERING, B. & MÜLLER, W.E. (1985). Interaction of piracetam with several neurotransmitter receptors in the central nervous system. *Arzneim-Forsch.*, **35,** 1350–1352.

BRUNI, J.F., VAN VUGT, D., MARSHALL, S. & MEITES, J. (1977). Effects of naloxone, morphine and methionine enkephalin on serum prolactin, luteinizing hormone, follicle stimulating hormone, thyroid stimulating hormone and growth hormone. *Life Sci.*, **21,** 461–466.

CHAUDHRY, H.R., DE MAHIEU, C., JAVAID, M.A. & CHAUDHRY, M.R. (1990). The effect of piracetam in the detoxification of heroin dependents. *Curr. Ther. Res.*, **48,** 313–319.

CHICHENKOV, O.N., KRYLOVA, I.N., BOROZDIN, M.Iu. & POKATILOV, D.V. (1990). [The influence of piracetam on the effects of narcotic analgesics]. *Farmakol. Toksikol.*, **53,** 22–24.

CHILDERS, S.R. (1991). Opioid receptor coupled second messenger systems. *Life Sci.*, **48,** 1991–2003.

COPANI, A., GENAZZANI, A.A., ALEPPO, G., CASABONA, G., CANONICO, P.L., SCAPAGNINI, U. & NICOLETTI, F. (1992). Nootropic drugs positively modulate α-amino-3-hydroxy-5-methyl-4-Isoxazolepropionic acid (AMPA)-sensitive glutamate receptors in neuronal cultures. *J. Neurochem.*, **58,** 1199–1204.

DENCKER, S.J. & WILHELMSON, G. (1978). Piracetam and chlormethiazole in acute alcohol withdrawal. A controlled clinical trial. *J. Int. Med. Res.*, **6,** 395–400.

GIURGEA, C.E. (1982). The nootropic concept and its prospective implications. *Drug Dev. Res.*, **2,** 441–446.

GOULIAEV, A.H. & SENNING, A. (1994). Piracetam and other structurally related nootropics. *Brain Res. Rev.*, **19,** 180–222.

HARMS, P.G. & OJEDA, S.R. (1974). A rapid and simple procedure for chronic cannulation of the rat jugular vein. *Life Sci.*, **38,** 987–992.

KORANYI, L. & ENDROCZI, E. (1983). Piracetam modifies morphine and related drug-induced elevation of serum corticosterone concentration in rats. *Acta Physiol. Hung.*, **62,** 75–83.

KRYLOVA, I.N., DROZD, Iu. V., NAUMOVA, V.I., IASNETSOV, V.V. & SHASHKOV, V.S. (1988). [Influence of piracetam on the effects of narcotic analgesics and opioid peptides]. *Farmakol. Toksikol.*, **51,** 42–45.

MACLEOD, R.M., JUDD, A.M., SPANGELO, B.L., ROSS, P.C., JARVIS, W.D. & LOGIN, I.S. (1988). Systems that regulate prolactin release. In *Prolactin Gene Family and its Receptors*. ed. Hoshimo, K. New York: Elsevier Science Publishers B.V. (Biomedical Division).

MARCHI, M., BESANA, E. & RAITERI, M. (1990). Oxiracetam increases the release of endogenous glutamate from depolarised rat hippocampal slices. *Eur. J. Pharmacol.*, **185,** 247–249.

MAREK, P., BEN-ELIYAHU, S., VACCARINO, A.L. & LIEBESKIND, J.S. (1991). Delayed application of MK-801 attenuates development of morphine tolerance in rats. *Brain Res.*, **558,** 163–165.

MATTON, A., BUYDENS, P., FINNE, E., GOVAERTS, J. & VANHAELST, L. (1991). Analysis of the receptor specificity of tolerance induction in stress versus opioid-related prolactin secretion in rats. *J. Endocrinol.*, **128,** 281–285.

MOYERSOONS, F. & GIURGEA, C. (1974). Protective effect of piracetam in experimental barbiturate intoxication : EEG behavioural studies. *Arch. Int. Pharmacodyn.*, **210,** 38–48.

NESTLER, E.J. (1992). Molecular mechanisms of drug addiction. *J. Neurosci.*, **12,** 2439–2450.

NICKOLSON, V.J. & WOLTHUIS, O.L. (1976). Effect of the acquisition-enhancing drug piracetam on rat cerebral energy metabolism. Comparison with naftidrofuryl and metamphetamine. *Biochem. Pharmacol.*, **25,** 2241–2244.

NIKOLOVA, M., NIKOLOV, R. & MILANOVA, D. (1984). Anti-hypoxic effect of piracetam and its interaction with prostacyclin. *Methods Find. Exp. Clin. Pharmacol.*, **6,** 367–371.

NYBACK, H., WIESEL, F.A. & SKETT, P. (1979). Effects of piracetam on brain monoamine metabolism and serum prolactin levels in the rat. *Psychopharmacology*, **61,** 235–238.

PIZZI, M., FALLACARA, C., ARRIGHI, V., MEMO, M. & SPANO, P.F. (1993). Attenuation of excitatory amino acid toxicity by metabotropic glutamate receptor agonists and aniracetam in primary cultures of cerebellar granule cells. *J. Neurochem.*, **61,** 683–689.

PUGLIESE, A.M., CORRADETTI, R., BALLERINI, L. & PEPEU, G. (1990). Effect of the nootropic drug oxiracetam on field potentials of rat hippocampal slices. *Br. J. Pharmacol.*, **99,** 189–193.

RAGAVAN, V.V. & FRANTZ, A.G. (1981). Opioid regulation of PRL secretion: evidence for a specific role of β-endorphin. *Endocrinology*, **109,** 1769–1771.

RAGO, L.K., ALLIKMETS, L.H. & ZARKOVSKY, A.M. (1981). Effects of piracetam on the central dopaminergic transmission. *Naunyn-Schmied. Arch. Pharmacol.*, **318,** 36–37.

RAITERI, M., COSTA, R. & MARCHI, M. (1992). Effects of oxiracetam on neurotransmitter release from rat hippocampus slices and synaptosomes. *Neurosci. Lett.*, **145,** 109–113.

RICHARDSON, A.E. (1977). Effect of piracetam on level of consciousness after neurosurgery. *Lancet*, **26,** 1110–1111.

SAMSON, W.K., McDONALD, J.K. & LUMPKIN, M.D. (1985). Naloxone-induced dissociation of oxytocin and prolactin releases. *Neuroendocrinology*, **40,** 68–71.

SATOH, M., ISHIHARA, K., IWAMA, T. & TAKAGI, H. (1986). Aniracetam augments, and midazolam inhibits, the long-term potentiation in guinea-pig hippocampal slices. *Neurosci. Lett.*, **68,** 216–220.

SIEGEL, R.A., CHOWERS, J., CONFORTI, N. & WEIDENFELD, J. (1982). Effects of naloxone on basal and stress-induced prolactin secretion, in intact, hypothalamic deafferented, adrenalectomized, and dexamethasone pretreated male rats. *Life Sci.*, **30,** 1691–1699.

SPIGNOLI, G., PEDATA, F., GIOVANELLI, L., BANFI, S., MORONI, F. & PEPEU, G. (1986). Effect of oxiracetam and piracetam on central cholinergic mechanisms and active-avoidance acquisition. *Clin. Neuropharmacol.*, **9,** Suppl.3, S39–S47.

TANG, C.M., SHI, Q.Y., KATCHMAN, A. & LYNCH, G. (1991). Modulation of the time course of fast EPSC's and glutamate channel kinetics by aniracetam. *Science*, **254,** 288–290.

TISEO, P.J. & INTURRISI, C.E. (1993). Attenuation and reversal of morphine tolerance by the competitive N-methyl-D-aspartate receptor antagonist LY274614. *J. Pharmacol. Exp. Ther.*, **264,** 1090–1096.

TOCCO, G., SHORS, T.J., BAUDRY, M. & THOMPSON, R.F. (1991). Selective increase of AMPA binding to the AMPA/quisqualate receptor in the hippocampus in response to acute stress. *Brain Res.*, **559,** 168–171.

TRUJILLO, K.A. & AKIL, H. (1991). Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. *Science*, **251,** 85–87.

TSUZUKI, K., TAKENCHI, T. & OZAWA, S. (1992). Agonist- and subunit-dependent potentiation of glutamate receptors by a nootropic drug aniracetam. *Mol. Brain Res.*, **16,** 105–110.

VAKULINA, O.P., IASNETSOV, V.V., ISACHENKOV, V.A., KRYLOVA, I.N., TISHKOVA, I.P., BRAGIN, E.O. & POPKOVA, E.V. (1990). [Effects of piracetam on pain sensibility and levels of β-endorphin in blood and cAMP in the cerebral cortex of rats]. *Biull. Eksp. Biol. Med.*, **109,** 163–165.

VAN VUGT, D.A., BRUNI, J.F. & MEITES, J. (1978). Naloxone inhibition of stress-induced increase in PRL secretion. *Life Sci.*, **22,** 85–90.

VERNON, M.W. & SORKIN, E.M. (1991). Piracetam. An overview of its pharmacological properties and a review of its therapeutic use in senile cognitive disorders. *Drugs Aging*, **1,** 17–35.

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