J. Pharm. Pharmacol. 1981, 33: 597–599
Communicated February 18, 1981

0022–3573/81/090597–03 $02.50/0

Naloxone antagonizes inhibitory and unmasks excitatory effects of baclofen

J. SAWYNOK*, F. S. LABELLA, *Department of Pharmacology and Therapeutics, University of Manitoba, Faculty of Medicine, Winnipeg, Manitoba, Canada R3E OW3*

Baclofen has been used clinically to reduce spasticity in various neurological disorders (Pinto et al 1972; Pedersen et al 1974). In rats, its administration directly into the brain produces analgesia, motor incoordination and hyperactivity (Levy & Proudfit 1979). Higher doses produce activation, ataxia, paddling, tail-pinch hyperresponse and anaesthesia (Smith & Vestergaard 1979). The effects of high doses, except for anaesthesia, are antagonized by agents which interfere with dopaminergic and noradrenergic function (Smith & Vestergaard 1979). Neurochemical studies suggest an involvement of monoaminergic mechanisms in baclofen action (Andén & Wachtel 1977; Waldmeier & Fehr 1978; Gianutsos & Moore 1978).

Baclofen enhances the release of methionine-enkephalin from brain slices (Sawynok & LaBella 1981a), but endogenous opiate release does not appear to mediate the analgesic action of baclofen because analgesia is not blocked by naloxone (Levy & Proudfit 1977; Sawynok & LaBella 1981b). However, symptoms of baclofen overdose (Paulson 1976) resemble those observed in opiate overdose (Jaffe & Martin 1975), suggesting endogenous opiate release could occur after high doses of baclofen. In the present study, the opiate antagonists naloxone and naltrexone were used to determine whether the endorphin system is involved in baclofen-induced anaesthesia. Endorphins induce anaesthesia through an action on opiate receptors (Havlicek et al 1980). In addition, because naloxone can act as an antagonist (Dingledine et al 1978) and baclofen as an agonist at certain GABA receptors (Bowery et al 1980; Sawynok & LaBella 1981a), the effects of known GABA antagonists were evaluated.

Male Swiss mice (27–33 g) were injected intraperitoneally and observed for gross behavioural responses and for loss and subsequent recovery of the righting reflex. Individual drugs were administered in a volume of 0·1 ml. Baclofen was suspended in propylene glycol —0·9% NaCl (saline) (50/50). Naloxone, naltrexone and picrotoxin were dissolved in saline, while bicuculline was suspended in saline and dissolved with the aid of 0·1 M HCl.

In mice, the intraperitoneal injection of baclofen in doses of up to 20 mg kg^{-1} has been reported to produce analgesia, muscle incoordination and hyperirritability (Levy & Proudfit 1977). In the present study, higher doses of baclofen (50–75 mg kg^{-1}) produced sedation and a hyperresponsiveness to innocuous stimuli (airpuff), followed by anaesthesia (loss of righting reflex, lack of response to foot- and tail-pinch). Injection of the propylene glycol–saline vehicle produced no observable behavioural changes. The time to onset and duration of anaesthesia observed in an initial series of experiments are shown in Table 1. High doses of naloxone (50 and 100 mg kg^{-1}) significantly delayed the onset of the loss of righting reflex

Table 1. Dose-related loss of righting reflex (RR) produced by baclofen in mice.

Dose baclofen (mg kg^{-1})	% showing loss of RR	Time to onset (min)	Duration (min)	No. of deaths in 4 h
50	57% (4/7)	12·8 ± 3·3	13·1 ± 5·6	1/7
60	83% (5/6)	10·4 ± 1·0	37·4 ± 17·8	1/6
75	100% (7/7)	9·5 ± 0·8	76·3 ± 24·7	4/7

produced by 60 mg kg^{-1} baclofen, but a lower dose (10 mg kg^{-1}) of the narcotic antagonist was ineffective (Fig. 1). There was considerable variability in the duration of anaesthesia, and significant effects of naloxone on duration of anaesthesia were not observed. The GABA antagonists picrotoxin (2 mg kg^{-1}) and bicuculline (2 mg kg^{-1}) injected immediately after baclofen had no effect on the induction of anaesthesia.

In a subsequent series of experiments, baclofen, 60 mg kg^{-1}, produced overt signs of excitation which included twitching of the hindlimbs and rotation along the longitudinal axis of the body (barrel rotation). In these mice, loss of the righting reflex did not occur until 15–25 min after injection of baclofen. Hypersalivation

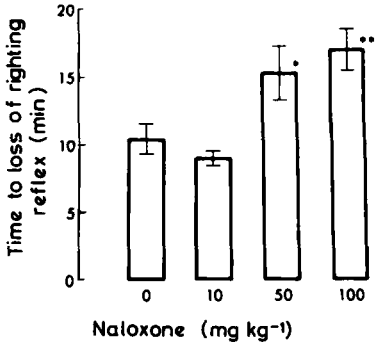

FIG. 1. Naloxone-induced delay in onset of loss of righting reflex produced by baclofen. Mice were injected with baclofen (60 mg kg^{-1}) followed by saline or naloxone. Columns represent the mean ± s.e.m. (n = 7). *P <0·05, **P <0·01 compared with saline-treated group (Duncan's multiple range test).

* Correspondence.

(foaming at the nose or mouth) occurred in some cases and appeared to contribute to an increased mortality rate. At 75 mg kg^{-1} drug, this excitability was no longer prominent. The effects of naloxone, picrotoxin, bicuculline and naltrexone on the time to loss of righting reflex and the incidence of barrel rotation are shown in Fig. 2. High doses of naloxone again prolonged the time to the loss of righting reflex and unmasked signs of excitability (twitching and barrel rolling). Many barrel rolls occurred rapidly in clusters so that counting was difficult, but of the order of 5–20 rotations were observed before the onset of the loss of righting reflex. In animals where hypersalivation was prominent, mortality was increased due to pulmonary congestion. Naltrexone (75 mg kg^{-1}), another narcotic antagonist produced similar effects to naloxone. Pretreatment with GABA antagonists did not alter the response to baclofen.

High doses of baclofen administered systemically produce behaviour with both excitatory and inhibitory components. Naloxone, in high doses, appears to antagonize the inhibitory component and unmask the excitatory component. Although high doses of naloxone have GABA antagonist activity at a bicuculline-sensitive site (Dingledine et al 1978), an alteration in GABA function is unlikely because bicuculline and picrotoxin are without effect on high doses of baclofen. Both naloxone and naltrexone antagonize baclofen-induced anaesthesia, suggesting a role for endogenous opiates in the induction of anaesthesia. However higher concentrations than those generally effective for narcotic antagonism are required to demonstrate antagonism of baclofen action, so it is possible that these effects are unrelated to antagonism at opiate receptors (see Sawynok et al 1979).

Many of the biological effects of baclofen are stereoselective for the (−)-isomer (Olpe et al 1978; Wilson & Yaksh 1978). Similarly, only the (−)-isomer produces a loss of the righting reflex in high doses (75 mg kg^{-1}, n = 6 mice/group). The effects of baclofen that are not stereoselective, such as an interaction with low affinity GABA receptors (Waddington & Cross 1979), and an increase in enkephalin release (Sawynok & LaBella 1981a), are unlikely to mediate this action. Some effects of baclofen that are stereoselective for the (−)-isomer may be due to an interaction with a novel GABA receptor (Bowery et al 1980), but other such effects are independent of GABA (Johnston et al 1980). In rats, the behavioural effects of high doses of baclofen administered centrally are stereoselective, but these effects are not mimicked by GABA (Smith & Vestergaard 1979).

The barrel rotations produced by baclofen in mice in the present study seem similar to those produced by somatostatin (Cohn & Cohn 1975), substance P (Rondeau et al 1978) and dynorphin (Herman et al 1980) administered centrally to rats. Whether barrel rotations produced by baclofen are mediated by an increased release of neuropeptides is unclear at present. Monoaminergic systems appear to be involved in some responses to high doses of baclofen (Smith & Vestergaard 1979), but it is likely that a number of neurotransmitter systems are involved.

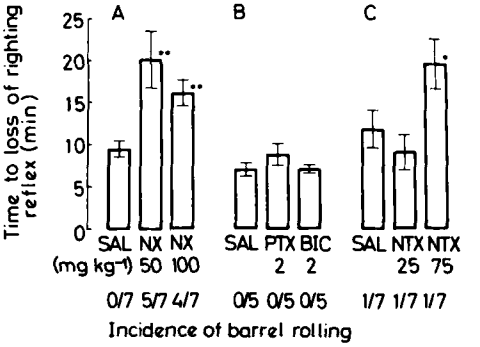

FIG. 2. Effects of naloxone (NX), picrotoxin (PTX), bicuculline (BIC) and naltrexone (NTX) on baclofen (75 mg kg^{-1}) injected into mice. Naloxone and naltrexone were injected immediately after baclofen, while picrotoxin and bicuculline were injected 60 min before baclofen. Panels represent separate experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with saline (SAL)-treated group (Duncan's multiple range test).

The authors are grateful to Ciba-Geigy for supplying baclofen and its isomers and Endo Laboratories for naloxone and naltrexone. This work was supported by the Medical Research Council of Canada and the St Boniface Hospital Research Foundation. J.S. is a Postdoctoral Fellow and F.S.L. a Career Investigator of the MRC.

REFERENCES

Andén, N.-E., Wachtel, H. (1977) Acta Pharmacol. Toxicol. 40: 310–320

Bowery, N. G., Hill, D. R., Hudson, A. L., Doble, A., Middlemiss, D. N., Shaw, J., Turnbull, M. (1980) Nature (London) 238: 92–94

Cohn, M. L., Cohn, M. (1975) Brain Res. 96: 138–141

Dingledine, R., Iversen, L. L., Breuker, E. (1978) Eur. J. Pharmacol. 47: 19–27

Gianutsos, G., Moore, K. E. (1978) J. Pharmacol. Exp. Ther. 207: 859–869

Havlicek, V., LaBella, F. S., Pinsky, C., Childiaeva (1980) Can. Anesth. Soc. J. 27: 535–539

Herman, B. H., Leslie, F., Goldstein, A. (1980) Life Sci. 27: 883–892

Jaffe, J. H., Martin, W. R. (1975) in: Goodman, L. S., Gilman, A. (eds) The Pharmacological Basis of Therapeutics. 5th edt., Macmillan, New York, pp 245–283

Johnston, G. A. R., Hailstone, M. H., Freeman, C. G. (1980) J. Pharm. Pharmacol. 32: 230–231

Levy, R. A., Proudfit, H. K. (1977) J. Pharmacol. Exp. Ther. 202: 437–445

Levy, R. A., Proudfit, H. K. (1979) Eur. J. Pharmacol. 57: 43–55

Olpe, H. R., Demiéville, H., Baltzer, V., Bencze, W. L., Koella, W. P., Wolf, P., Haas, H. L. (1978) Eur. J. Pharmacol., 52: 133–136

Paulson, G. W. (1976) Neurology 26: 1105–1106

Pedersen, E., Arlien-Soborg, P., Mai, J. (1974) Acta Neurol. Scand. 50: 665–680

Pinto, O. de S., Polikar, M., Debono, G. (1972) Postgrad. Med. J. Suppl. 48: 18–23

Rondeau, D. B., Jolicoeur, F. B., Belanger, F., Barbeau, A. (1978) Pharmacol. Biochem. Behav. 9: 769–775

Sawynok, J., LaBella, F. S. (1981a) Eur. J. Pharmacol. 70: 103–110

Sawynok, J., LaBella, F. S. (1981b) Neuropharmacol. in the press

Sawynok, J., Pinsky, C., LaBella, F. S. (1979) Life Sci. 25: 1621–1632

Smith, D. F., Vestergaard, P. (1979) J. Neural Trans. 46: 215–223

Waddington, J. L., Cross, A. L. (1979) Neurosci. Lett., 14: 123–127

Waldmeier, P. C., Fehr, B. (1978) Eur. J. Pharmacol. 49: 177–184

Wilson, P. R., Yaksh, T. L. (1978) Ibid. 51: 323–330

J. Pharm. Pharmacol. 1981, 33: 599–600
Communicated December 5, 1980

0022–3573/81/090599–02 $02.50/0

Naloxone reverses reserpine-induced hypokinesia in rats

KRISTOFFER HELLSTRAND, MIKAEL DELLBORG*, *Department of Pharmacology, University of Göteborg, Box 33031, S-400 33 Göteborg, Sweden*

An increasing number of reports indicate interactions between endogenous opioid systems and catecholaminergic neurotransmission in the central nervous system (for review, see Iwamoto & Way 1979). For example, morphine produces behavioural effects in the rat similar to those of neuroleptic drugs, i.e. hypokinesia, catalepsy and rigidity. These effects are accompanied by an increase in the turnover of brain dopamine and an increase in the firing rate of dopamine-containing cells in the substantia nigra (for review, see Kuschinsky 1976). All these effects are readily reversed by the opiate receptor antagonist naloxone, indicating that they are mediated by opiate receptors. Naloxone-induced reversal of morphine-induced hypokinesia led us to examine effects of naloxone on hypokinesia induced also by other drugs. We now report an interaction between reserpine, which causes hypokinesia thought to be secondary to depletion of brain catecholamines (for review, see Carlsson 1965) and naloxone.

Materials and methods

Male Sprague-Dawley rats (Anticimex) 220–280 g were used. Locomotor activity was recorded in a circular open-field arena with a diameter of 75 cm surrounded by 40 cm high cylinder as previously described by Engel et al (1975). Locomotor activity was recorded for 45 min.

The following drugs were used, alone or in combinations: reserpine (Ciba), naloxone HCl (Endo), α-methyl-*p*-tyrosine HCl (AMPT, Hässle), haloperidol (Leo), and phenoxybenzamine HCl (S K & F). Reserpine was used from the commercially available vial (Serpasil). Naloxone and AMPT were dissolved in 0·9% NaCl (saline). Phenoxybenzamine was suspended in saline and subsequently gently heated. Haloperidol was dissolved in a few drops of glacial acetic acid and subsequently diluted in 5·5% glucose solution. All drugs were injected i.p.

Statistical significance was calculated by Student's *t*-test. *P*-values higher than 0·05 were considered not significant.

Results

All rats were pretreated with reserpine (10 mg kg^{-1}) 6 h before testing. Controls, injected with 0·5 ml of saline immediately before being placed in the open-field arena, exhibited throughout the 45 min test the typical reserpine syndrome, i.e. catalepsy, rigidity, ptosis, a hunched back and little spontaneous locomotor activity (activity score 70 ± 21, n = 9, see Fig. 1). Rats injected with naloxone (5 mg kg^{-1}) immediately before testing showed a marked increase in locomotor activity (activity score 557 ± 125, n = 13, see Fig. 1). These rats exhibited no obvious signs of sniffing, gnawing or licking during the test; they walked or ran, preferably along the walls of the arena, starting 2–3 min after the naloxone injection. This locomotor stimulation persisted throughout the test. The open-field activity score of this group was significantly different from that of the reserpine-treated control rats ($P < 0.005$). AMPT, a catecholamine synthesis inhibitor (250 mg kg^{-1}), injected 4 h after the administration of reserpine, prevented the naloxone-induced icnrease in locomotor activity (activity score 54 ± 13, n = 10, see Fig. 1). Thus, the activity score of this group was significantly reduced compared with that of rats receiving reserpine + naloxone

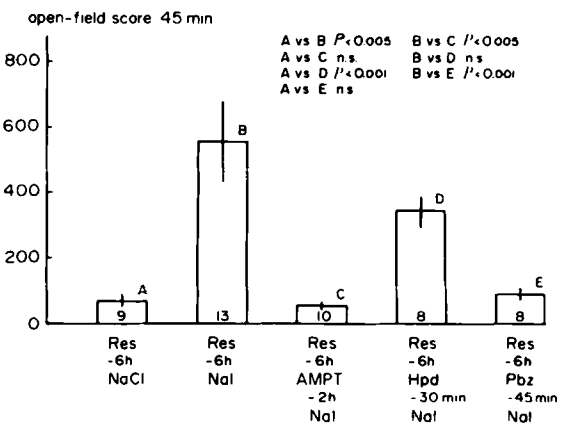

FIG. 1. Effects of α-methyl-*p*-tyrosine (AMPT; 250 mg kg^{-1}), haloperidol (Hpd; 2 mg kg^{-1}) and phenoxybenzamine (Pbz; 10 mg kg^{-1}) on naloxone- (Nal; 5 mg kg^{-1}) induced locomotor stimulation in rats pretreated with reserpine (Res; 10 mg kg^{-1}). Naloxone and NaCl were administered immediately before the open-field test. Number of subjects in each experiment is indicated in the bars. Statistical significance was calculated by Student's *t*-test.

* Correspondence.