Naunyn-Schmiedeberg's Arch Pharmacol (1987) 335:154–159

Naunyn-Schmiedeberg's
Archives of
Pharmacology

The role of descending inhibition in the antinociceptive effects of the pyrazolone derivatives, metamizol (dipyrone) and aminophenazone ("Pyramidon")*

Karl-Heinz Carlsson and Ilmar Jurna

Institut für Pharmakologie und Toxikologie der Universität des Saarlandes, D-6650 Homburg/Saar, Federal Republic of Germany

Summary. The study was carried out to provide further evidence that the two pyrazolone derivatives, metamizol and aminophenazone, produce central antinociceptive effects by stimulating inhibition descending from the periaqueductal grey (PAG) to the spinal cord. Experiments were carried out on rats in which the tail-flick response to radiant heat, nociceptive activity in ascending axons of the spinal cord, and activity of neurones in the PAG and the substantia nigra were studied. Microinjection of procaine (10 µg) into the PAG reduced the tail-flick latency and abolished the increase in latency caused by i.p. injection of metamizol (40 mg/kg) and aminophenazone (150 mg/kg); it did not significantly reduce the antinociceptive effect of i.p. injection of morphine (2 mg/kg). Threshold doses of morphine (1 and 2 µg) administered by intrathecal (i.t.) injection potentiated the effect of threshold doses of metamizol injected i.p. (10 mg/kg) or into the PAG (10 µg) in the tail-flick test. Morphine (2 µg) injected i.t. potentiated the effect of i.v. injection of metamizol (80 mg/kg) on nociceptive activity in ascending axons by eliminating the stimulant effect of metamizol on about one third of the axons. Threshold doses of morphine injected i.t. failed to potentiate the antinociceptive effect of aminophenazone (50 mg/kg) injected i.p. in the tail-flick test. The results support the view that metamizol and aminophenazone activate pathways descending from the PAG and exerting an inhibitory effect on nociceptive impulse transmission at the spinal level.

Key words: Tail-flick – Ascending nociceptive activity – Periaqueductal grey – Substantia nigra – Metamizol – Aminophenozone – Morphine – Procaine

Introduction

It is now well established that morphine exerts its antinociceptive and analgesic effects in part by activating spinal inhibition descending from the periaqueductal grey (PAG; Yaksh and Rudy 1978; Gebhart 1982), and it seems that other analgesic agents which are non-opioid in nature may also exhibit such action. Very recently, the pyrazolone derivative, metamizol, has been demonstrated to depress the tail-flick response and nociceptive activity in ascending axons of the spinal cord in rats when it was administered by microinjection into the PAG (Carlsson et al. 1986a).

* Supported by the Schwerpunkt „Nociception und Schmerz" of the Deutsche Forschungsgemeinschaft
Send offprint requests to I. Jurna at the above address

Stimulation by metamizol of pathways which descend from the PAG and cause inhibition of impulse transmission in spinal nociceptive circuits is probably due to a central excitatory action which this drug shares, although to a lesser degree, with other pyrazolone derivatives. Aminophenazone ("Pyramidon") is known to elicit seizure activity (Starkenstein et al. 1934; Driesen et al. 1950). Aminophenazone and isopropylaminophenazone were found to increase reflex activity in spinal cats and to depress it in animals in which the spinal cord was under control of the brain stem (Jurna 1963) which suggested that these drugs not only produce excitation but also can activate inhibitory mechanisms in the central nervous system. Similarly, aminophenazone was found to facilitate the tail-flick response at the spinal level, while it depressed the response in rats with an intact central nervous system (Carlsson et al. 1986b).

The present study was designed to provide further evidence that the central antinociceptive and analgesic effects of metamizol and aminophenazone result from activation of an inhibitory output from the brain stem. For this purpose, experiments were carried out on rats in which the tail-flick response, nociceptive activity in ascending axons of the spinal cord, and activity of single neurones in the PAG and substantia nigra were studied. Activation of inhibitory pathways originating in the PAG and depressing nociceptive impulse transmission within the spinal cord was blocked by microinjection of procaine in this brain stem area, and the spinal excitatory effects of the two pyrazolone derivatives were prevented by intrathecal (i.t.) injection of morphine.

Methods

General procedure. The experiments were carried out on rats of either sex (Sprague Dawley/SIV; 250 g – 300 g body weight). The animals were housed in macrolon cages (six animals per cage) and received a standard diet (Altromin) and tap water ad libitum. For i.v. injections, a catheter was inserted into a tail vein. The volume administered by i.v. or i.p. injection was 0.8 ml/kg. For i.t. injection, a polyethylene catheter (outer diameter 0.4 mm) was inserted into the lumbosacral subarachnoid space. I.t. administration of drugs was made injecting a volume of 5 µl saline at a rate of 20 µl/min. The catheter was rinsed by subsequent injection of 5 µl saline. Sham injections were made by administering saline 0.8 ml/kg i.p. or i.v. or two times 5 µl/rat i.t. The position of the catheter was checked by injecting Evans blue after the end of the experiments.

For i.t. injection to rats that were used in the tail-flick test, a catheter was inserted under ether anaesthesia into the

subarachnoid space according to the method described by Yaksh and Rudy (1976a). Surgery and recording activity in ascending spinal axons was carried out after a single i.p. injection of urethane (1.2 g/kg). The sleeping time assessed by the righting reflex in animals treated with this dose of urethane was 6.5 h (Carlsson et al. 1986a). Significances of differences were established by applying Student's *t*-tests for paired or unpaired samples.

Tail-flick test. The experiments were carried out from 09:00 to 12:00 a.m. The latency of the tail-flick response to radiant heat was measured in two groups each of ten rats. The time of exposure to noxious heat was unlimited. The animals of one group received a sham injection of saline, and those of the other group were injected with a drug or a combination of drugs. When procaine was administered by microinjection in the PAG, sham injection was also made in the PAG. The interval between injection and test was 20 min for metamizol i.p., 30 min for aminophenazone i.p., 30 min for morphine i.p., 10 min for morphine i.t., and 10 min for procaine injected into the PAG. Each animal was tried only once. The effects of i.t. injections were determined 2 days after implantation of the catheter.

Nociceptive activity in ascending axons. The procedure to record activity in ascending axons following i.t. drug injection has been described elsewhere (Doi and Jurna 1982). In the present experiments, the brain and spinal cord were left intact and the animals were kept under urethane anaesthesia. The left sural nerve was prepared for stimulation with bipolar platinum wire electrodes. The spinal cord was exposed from Th_8 to L_2 by laminectomy. The catheter used for i.t. injections was introduced into the lumbosacral subarachnoid space through the opening made by laminectomy. The exposed cord was covered with agar which sealed the spinal canal and fixed the catheter. A window was cut into the agar to give the microelectrode access to the spinal cord. The exposed spinal cord was covered by warm paraffin oil kept at 37° C by radiant heat. Potentials in ascending axons were recorded on the left side at the level of $Th_{10}-L_1$ using tungsten microelectrodes (tip diameter 1 µm, resistance 1 ΩM). On termination of surgery, the animals were immobilized by gallamine triethiode and artificially ventilated. Body temperature was monitored in the rectum and kept between 37.5° C and 38° C by radiant heat.

The sural nerve was stimulated by single rectangular impulses at a frequency of 0.3 Hz and a duration of 0.05 ms. Stimulation strength was supramaximal for afferent C fibres.

Recording from neurones in the PAG and the substantia nigra and microinjection into the PAG. Spontaneous activity of single neurones in the brainstem was recorded with tungsten microelectrodes (tip diameter 1 µm; resistance 10 M) mounted to a micromanipulator which was fixed to a stereotaxic device. For recording from the PAG, the electrode was positioned at the co-ordinates AP: 6.0 mm, L: 0.0–0.5 mm and V: 6.00 mm, and for recording from the substantia nigra the co-ordinates were AP: 4.5 mm, L: 2.0 mm and V: 8.0 mm according to the atlas of Fifková and Maršala (1967). Microinjections were made at the same co-ordinates as described previously (Jurna and Zetler 1981). For this purpose, a steel cannula (outer diameter 0.4 mm) was inserted into a guide cannula (outer diameter 0.7 mm). The tip of the injection cannula protruded 0.5 mm from the orifice of the guide. For implantation, the rats were anaesthetized with ether. The guide cannula was fixed to the skull with dental cement. Injections were made on the left side. Procaine (10 µg) and metamizol (10 µg) were administered in a total volume of 0.2 µl (injection rate 1 µl/min) 10 min or 15 min, respectively, before the test. Sham injection was carried out with 0.2 µl saline. Evans blue dye was injected after the end of the experiment and standard histological procedures employed to assure correct position of the cannula.

Recording and evaluation of potentials. Potentials from ascending axons and spontaneously discharging neurones in the PAG and the substantia nigra were amplified, displayed on a cathode ray oscilloscope, recorded on tape and evaluated using an averaging computer (Nicolet Instrument Model 1072; number of computer addresses 1024). Twenty consecutive responses to sural nerve stimulation were summed each time. Activity recorded from single neurones in the PAG or substantia nigra were evaluated in sections of 100 s duration. Frequency histograms were plotted and electronically integrated. The integrations were pooled for statistical evaluation. Four to six determinations were made before drug administration and served as controls. Metamizol or aminophenazone were administered only once in each experiment. Therefore, the number of ascending axons or neurones in the brain stem recorded from, the number of rats used and the number of experiments carried out are identical.

Drugs. The drugs used were aminophenazone (Hoechst, Frankfurt, FRG), diethylether (Asid Bonz, Böblingen, FRG), gallamine triethiodide (Flaxedil, Abbott, Ingelheim, FRG), metamizol sodium (Novalgin, Hoechst, Frankfurt, FRG), morphine hydrochloride (Merck, Darmstadt, FRG), naloxone hydrochloride (Narcanti, Endo Laboratories, Brussels, Belgium), pentobarbital (Nembutal, Abbott, Ingelheim, FRG), procaine (Novocain, Hoechst, Frankfurt, FRG), and urethane (Riedel-De Haen, Seelze, FRG).

Results

Tail-flick response

The intensity of noxious radiant heat applied to the tail of rats was chosen so as to elicit a withdrawal response with a latency of about 5 s after sham injection of saline. Metamizol (40 mg/kg) injected i.p. 20 min before the test increased the tail-flick latency (Fig. 1 a). This dose of metamizol had been found to produce an antinociceptive effect in 90% of the animals tested (Carlsson et al. 1986a). Microinjection of the local anaesthetic agent, procaine (10 µg), into the PAG 10 min before the test reduced the latency (Fig. 1 a). When metamizol and procaine were given in combination, metamizol failed to increase the latency (Fig. 1 a). This result agrees with the view that metamizol produces its antinociceptive effect by stimulating spinal inhibition descending form the PAG.

Aminophenazone (150 mg/kg) injected i.p. 30 min before the test prolonged the tail-flick latency, and the antinociceptive effect of the drug was abolished by microinjection of procaine into the PAG (Fig. 1 b). In a previous investigation (Carlsson et al. 1986b), the dose of

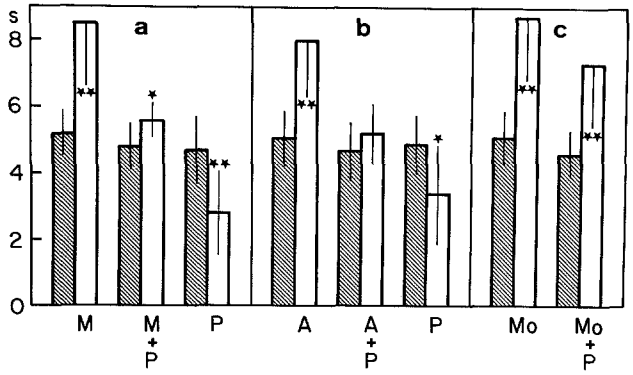

Fig. 1a–c. Effect of procaine microinjected into the PAG on the antinociceptive effects of metamizol, aminophenazone and morphine in the tail-flick test. The columns present the mean values determined in groups of ten rats. *Hatched columns:* sham injection (controls). *Open columns:* drug injection. The vertical bars at the top of the columns are SD. The scale on the left indicates the tail-flick latency in seconds. Procaine (*P*; 10 µg) was injected into the PAG 10 min before the test in **a–c**. Metamizol (*M*; 40 mg/kg) was injected i.p. 20 min before the test in **a**. Aminophenazone (*A*; 150 mg/kg) was injected i.p. 30 min before the test in **b**. Morphine (*Mo*; 2 mg/kg) was injected i.p. 30 min before the test. *Asterisks* indicate that values differ significantly from the respective control values ($*P < 0.05$; $**P < 0.01$)

aminophenazone employed here had been found to cause maximum depression of the tail-flick response; the ED_{50} of the antinociceptive effect of aminophenazone administered by i.p. injection was 107 (90.7–126.3) mg/kg, the values in brackets indicating the 95% confidence limits. Moreover, it had been observed earlier that aminophenazone facilitated the tail-flick response at the spinal level. When it was administered by i.p. injection to rats in which the spinal cord was acutely transected, it reduced the tail-flick latency; the ED_{50} of the facilitated response was 46.5 (32.1–67.4) mg/kg. Likewise, i.t. injection of aminophenazone facilitated the nociceptive reflex, the ED_{50} being 265 (193.4–363) µg. Consequently, abolition of the antinociceptive effect of aminophenazone caused by microinjection of procaine in the PAG indicates that aminophenazone depresses nociceptive reflex activity by stimulating inhibition descending from the brain stem.

Microinjection of procaine in the PAG did not significantly reduce the antinociceptive effect of morphine (2 mg/kg) injected i.p. 30 min before the test (Fig. 1c). The dose of morphine used had previously produced an antinociceptive effect in 65% of the animals tested, the ED_{50} of morphine injected i.p. being 1.3 (0.8–1.9) mg/kg. The observation that procaine microinjected into the PAG fails to markedly reduce morphine-induced depression of the tail-flick response suggests that stimulation of descending inhibition is not as important as the spinal antinociceptive action of morphine in this test.

The threshold dose of the antinociceptive effect of morphine administered by i.t. injection has previously been determined to be 2 µg (Jurna and Zetler 1981). The i.p. threshold dose of metamizol is 10 mg/kg (Carlsson et al. 1986a), and that of aminophenazone is about 50 mg/kg (Carlsson et al. 1986b). In the present study, neither morphine administered by i.t. injection at doses of 1 and 2 µg 10 min before the test (Fig. 2a) nor metamizol (10 mg/kg; Fig. 2b) or aminophenazone (50 mg/kg; Fig. 2c) injected i.p. caused a change in the tail-flick latency. When

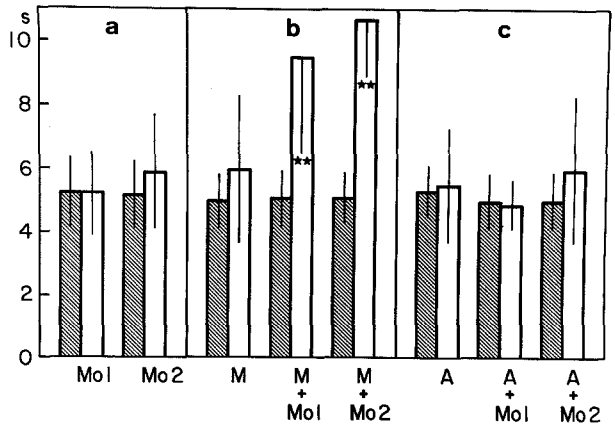

Fig. 2a–c. Effect of threshold doses of morphine injected i.t. on the effect of i.p. threshold doses of metamizol and aminophenazone in the tail-flick test. Morphine (*Mo1* and *Mo2*; 1 µg and 2 µg) was injected i.t. 10 min before the test in **a–c**. Metamizol (*M*); 10 mg/kg) was injected i.p. 20 min before the test in **b**. Aminophenazone (*A*; 50 mg/kg) was injected i.p. 30 min before the test in **c**. Other details as in Fig. 1

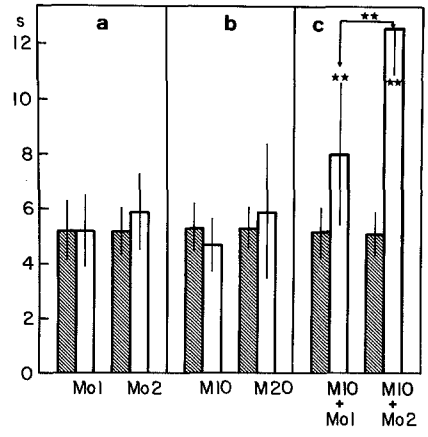

Fig. 3a–c. Effect of threshold doses of morphine injected i.t. on the effect of a threshold dose of metamizol microinjected into the PAG. Morphine (*Mo1* and *Mo2*; 1 µg and 2 µg) was injected i.t. 10 min before the test in **a–c**. Metamizol (*M10* and *M20*; 10 µg and 20 µg) was injected into the PAG 15 min before the test in **b** and **c**. *Asterisks* indicate that the values differ significantly from the respective controls or, when in bracket, that the two values after drug injection are significantly different ($**P < 0.01$)

metamizol was administered in combination with morphine 1 µg or 2 µg, the latency was markedly increased (Fig. 2b). This means that morphine injected i.t. potentiated the antinociceptive effect of metamizol injected i.p. However, no increase in the latency occurred after a combined administration of morphine and aminophenazone (Fig. 2c).

Metamizol administered by microinjection in the PAG at the previously determined threshold doses 10 µg and 20 µg (Carlsson et al. 1986a) 15 min before the test, caused no change in tail-flick latency (Fig. 3b). When metamizol (10 µg) was injected into the PAG together with an i.t. injection of morphine (1 µg or 2 µg), the latency was increased in dependence of the dose of morphine employed (Fig. 3c). The combination of metamizol with the high dose of morphine was significantly more effective than the combination of metamizol with the low dose. These results demonstrate, again, that the spinal antinociceptive action of

morphine potentiates the supraspinal antinociceptive action of metamizol.

Due to the low solubility of aminophenazone in water it was not possible to apply this drug by microinjection in the PAG in threshold amounts for producing an antinociceptive effect. Therefore, the effect of administration of metamizol in the PAG in combination with threshold doses of morphine applied to the spinal cord could not be studied.

Nociceptive activity in ascending axons

Morphine applied into the subarachnoid space by i.t. injection and metamizol microinjected into the PAG potentiated each other in their antinociceptive effects in the tail-flick test (Fig. 3b). It might be of practical importance to demonstrate that the combination of the spinal action of morphine and the supraspinal action of metamizol also cause a potentiated depression of nociceptive activity in ascending axons of the spinal cord, because ascending nociceptive activity elicits pain sensation when arriving in the thalamus, and its depression is an equivalent for analgesia in man (Doi and Jurna 1981).

Metamizol administered by i.v. injection to rats with intact brain and spinal cord had been found to reduce nociceptive activity in most ascending axons studied, but in some axons it increased the activity (Carlsson et al. 1986a). Although depression of ascending nociceptive activity prevailed after administration of metamizol at doses of 40 mg/kg, 80 mg/kg and 140 mg/kg, it was impossible to establish a dose-response relationship of the depressant effect because of the stimulant effect of the drug on some of the axons. Therefore, metamizol was employed in the present experiments at a dose of 80 mg/kg.

The interaction between morphine (2 μg) injected i.t. and metamizol (80 mg/kg) injected i.v. was determined in 20 ascending axons responding to electrical stimulation of afferent C fibres in the ipsilateral sural nerve. All axons exhibited co-activation from afferent Aδ fibres (Jurna and Heinz 1979). Morphine was employed in all experiments. Metamizol was used in experiments carried out on eight axons, and sham injection with saline administered i.v. instead of metamizol was made in experiments on twelve axons. Metamizol or saline were injected 20 min after i.t. injection of morphine. Metamizol reduced spontaneous and C fibre-evoked activity as compared to the values after morphine injection or the corresponding values after sham injection (not shown). Pretreatment with the threshold dose of morphine did not increase the mean depressant effect of metamizol as compared to the values obtained after i.v. injection of metamizol (80 mg/kg) alone in previous experiments (Carlsson et al. 1986a). The depression of spontaneous activity from 20 min to 30 min after metamizol alone amounted to 50% of the control values, and the respective depression caused by metamizol administered after injection of morphine was 40%, the difference between both values not being significant. Depression of C fibre-evoked activity was 30% in both series of experiments. However, in no axon was the nociceptive activity found to be increased when the injection of metamizol was preceded by an administration of morphine to the spinal cord. Thus, the spinal depressant effect of morphine blocks the spinal excitatory effect of metamizol so that the number of axons exhibiting a depressant effect of metamizol is increased.

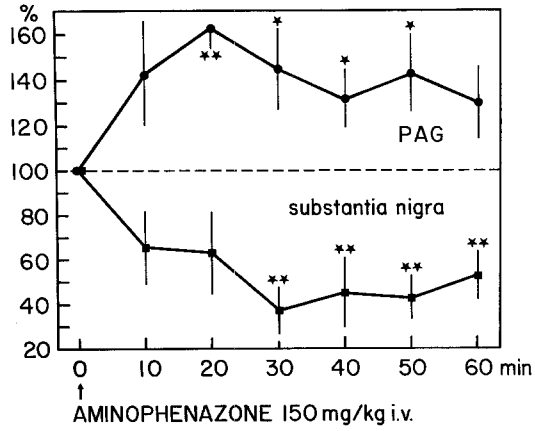

Fig. 4. Effects of aminophenazone on spike activity of single neurones in the PAG and substantia nigra. *Ordinate:* change in activity in per cent of controls. *Abscissa:* time in minutes after i.v. injection of aminophenazone (150 mg/kg). Each point on the curves is the mean value determined in seven out of eight neurones in the PAG (upper curve), and in five out of eight neurones in the substantia nigra (lower curve). The *vertical lines* in the points present SEM. *Asterisks* indicate that the values differ significantly from the controls (*$P < 0.05$; **$P < 0.01$). Rats under urethane anaesthesia

Since threshold doses of morphine did not potentiate the effect of aminophenazone in the tail-flick test and aminophenazone increasd the mean C fibre-evoked activity in ascending axons (Carlsson et a. 1986b), the interaction of morphine and aminophenazone on ascending nociceptive activity was not studied.

Activity in the PAG and the substantia nigra

Metamizol has recently been reported to increase the activity of single neurones in the PAG and to reduce that of neurones in the substantia nigra (Carlsson et al. 1986a). This was considered as evidence that metamizol activates inhibition descending to spinal nociceptive pathways. In the present investigation, it was aimed to verify that also aminophenazone produces an antinociceptive effect by stimulating neurones in the PAG.

Aminophenazone (150 mg/kg) injected i.v. increased the activity of seven neurones out of eight recorded from in the PAG (Fig. 4). The effect was significant from 20 min to 50 min after injection. The discharge rate of the remaining neurone was not markedly altered by aminophenazone. This result is essentially the same as the one obtained when employing metamizol and indicates that aminophenazone activates spinal inhibition descending from the PAG.

For comparison, activity was recorded from single neurones in the substantia nigra which is also involved in the processing of nociceptive signals (see Discussion). Aminophenazone (150 mg/kg) injected i.v. reduced the activity in five out of eight neurones (Fig. 4). The effect was significant from 30 min after injection till the end of the experiment at 60 min after injection. Activity was not significantly changed in the remaining three neurones. A similar observation has been made after injection of metamizol (Carlsson et al. 1986a).

Discussion

Recent observations indicate that metamizol depresses the tail-flick response and nociceptive activity in axons ascend-

ing in the spinal cord by stimulating spinal inhibition descending from the PAG (Carlsson et al. 1986a). In this respect the action of metamizol resembles that of morphine (Yaksh and Rudy 1978; Gebhart 1982; Gebhart et al. 1983; Jurna and Zetler 1985) and electrical stimulation in the PAG (Reynolds 1969; Mayer et al. 1971; Balagura and Ralph 1973; Lewis and Gebhart 1977; Jurna 1980). It is in agreement with this view that the depression of the tail-flick response caused by systemically administered metamizol is abolished after microinjection of procaine in the PAG. The reduction in tail-flick latency caused by procaine alone signals elimination of descending inhibition. Similarly, microinjection of lidocaine in the nucleus raphe magnus and medullary reticular formation has been reported to block descending inhibition of nociceptive neurones in the dorsal horn of the cat spinal cord (Gebhart et al. 1983). Thus, the result of microinjecting procaine into the PAG is the same as that obtained in the tail-flick test after acute transection of the spinal cord (Jurna 1972), i.e. facilitation of the tail-flick response due to elimination of inhibition from the brain stem. Since aminophenazone is poorly soluble in water, it could not be administered by microinjection in the PAG in amounts sufficient to decide whether or not it activates descending inhibition as does metamizol. However, indirect evidence for such action is provided by the observation that microinjection of procaine in the PAG abolished the depressant effect exerted by systemically administered aminophenazone in the tail-flick test. This indicates that the antinociceptive effect of aminophenazone exclusively derives from activation of inhibition descending from the PAG. In spinal rats, aminophenazone facilitated the nociceptive reflex response in a dose-dependent way (Carlsson et al. 1986b) due to its spinal excitatory action (Jurna 1963). Thus, normally, activation of descending inhibition must be so powerful that it surmounts the spinal excitatory effect. At no dose of aminophenazone could a depressant effect be detected in the tail-flick test performed on spinal rats as should have been the case if the drug exerted an action in the periphery. This agrees with the finding that this drug does not inhibit prostaglandin synthesis in the in vitro experiment (Brune et al. 1981). In this respect, it differs from metamizol, whose main metabolites, i.e. 4-methylaminoantipyrine and 4-aminoantipyrine, have been reported to inhibit the formation of prostaglandins as effectively as acetylsalicylic acid (Weithman and Alpermann 1985). In contrast to aminophenazone, metamizol does not facilitate the tail-flick response by a spinal action in the range of doses which prolong the response in intact rats after systemic administration (Carlsson et al. 1986a).

Interestingly, procaine injected into the PAG did not markedly reduce the depressant effect of morphine in the tail-flick test, suggesting that the spinal action of the drug in this test (Jurna 1972; Yaksh and Rudy 1976b) prevails over the supraspinal action. This result apparently is at variance with the observations of Yeung and Rudy (1980) that morphine given by the intraventricular or the i.t. route alone produced a full antinociceptive effect in the tail-flick test, and that the optimal balance between spinally and supraspinally administered doses in producing potentiation of the antinociceptive effect was achieved when both doses were equal. However, it must be taken into account that in the present study procaine was applied locally to the PAG so that not all neurones giving rise to morphine-induced inhibition were affected by the local anaesthetic agent.

Other indirect evidence for stimulation by aminophenazone of supraspinal inhibition derives from the result that aminophenazone increased the activity of neurones in the PAG. Such activation was also seen after administration of metamizol (Carlsson et al. 1986a) and would also result form electrical stimulation in this brain stem area producing inhibition of spinal nociceptive activity.

In contrast to its stimulant effect on neurones in the PAG, aminophenazone reduced the activity of neurones in the substantia nigra (Pay and Barasi 1982). This effect may also be involved in aminophenazone-induced antinociception and analgesia, because neurones in the zona compacta and zona reticulata of the substantia nigra are activated by noxious stimuli (Barasi 1979) and project to the thalamus. Moreover, microinjection of procaine into the substantia nigra depressed the tail-flick response in rats (Jurna et al. 1978).

Threshold doses of morphine administered directly to the spinal cord potentiated the effect of a systemically administered threshold dose of metamizol administered either systemically or by microinjection in the PAG. It therefore seems that depression of impulse transmission from nociceptive afferents caused by morphine at the spinal level (Zieglgänsberger 1985) and descending inhibition from the brain stem activated by metamizol interact at neurones of the nociceptive system in the spinal cord in a way that leads to a supra-additive effect. Similarly, it has been observed that clonidine potentiated the antinociceptive effect of morphine when both drugs were administered by i.t. injection at threshold doses (Wilcox et al. 1986). The potentiating effect of clonidine has been interpreted in terms of imitation by this drug of the effect of the inhibitory transmitter noradrenaline released from the terminals of descending axons in the spinal cord.

Pretreatment with a threshold dose of morphine administered by i.t. injection did not increase the depressant effect of metamizol on nociceptive activity in ascending axons, but it blocked the excitatory effect of metamizol that had been observed in about one third of the axons recorded from in a previous investigation (Carlsson et al. 1986a). This means that after administration of metamizol the input on the thalamus of nociceptive activity in a large number of ascending axons will be less when morphine acts simultaneously on the spinal cord than in its absence. Thus, morphine potentiates the effect of metamizol not only on the motor response but also on the sensory response of the spinal nociceptive system.

Contrary to the observation made after administration of metamizol, threshold doses of morphine injected i.p. did not render a threshold dose of aminophenazone effective in depressing the tail-flick response. The two phenazone derivatives differ in their spinal action. Aminophenazone dose-dependently facilitates the nociceptive reflex, the dose employed in the present experiments (50 mg/kg i.p.) reducing the tail-flick latency in about 20% of the animals (Carlsson et al. 1986b), while metamizol is devoid of such facilitatory effect (Carlsson et al. 1986a). It is therefore possible that the stimulant effect of aminophenazone at the spinal level prevents morphine from potentiating a supraspinal antinociceptive effect of aminophenazone.

In conclusion it can be stated that the results provide further evidence that metamizol produces a central antinociceptive and analgesic effect by stimulating spinal inhibition from the PAG. Moreover, they show that the

supraspinal activation by metamizol of descending pathways producing inhibition of nociceptive activity in the spinal cord is potentiated by the spinal inhibitory action of morphine. Finally, they reveal that the antinociceptive effect of aminophenazone is due to stimulation of descending inhibition counteracting a potent excitatory of the drug at the spinal level.

Acknowledgements. The authors are indebted to Mrs. Birgit Spohrer, Miss Hannelore Bleyer and Mr. Hans-Jürgen Erkel for technical assistance.

References

Balagura S, Ralph T (1973) The analgesic effect of electrical stimulation of the diencephalon and mesencephalon. Brain Res 60:369–379

Barasi S (1979) Responses of substantia nigra neurones to noxious stimulation. Brain Res 171:121–130

Brune K, Rainsford KD, Wagner K, Peskar BA (1981) Inhibition by anti-inflammatory drugs of prostaglandin production in cultured macrophages. Naunyn-Schmiedeberg's Arch Pharmacol 315:269–276

Carlsson K-H, Helmreich J, Jurna I (1986a) Activation of inhibition from the periaqueductal grey matter mediates central analgesic effect of metamizol (dipyrone). Pain (in press)

Carlsson K-H, Helmreich J, Jurna I (1986b) Comparison of central antinociceptive and analgesic effects of the pyrazolone derivatives, metamizol (dipyrone) and aminophenazone („Pyramidon"). Schmerz-Pain-Douleur 7:93–100

Doi T, Jurna I (1982) Analgesic effect of intrathecal morphine demonstrated in ascending nociceptive activity in the rat spinal cord and ineffectiveness of caerulein and cholecystokinin octapeptide. Brain Res 234:399–407

Driesen W, Hahn F, Rummel W (1950) Über den Einfluß von Cardiazol, Coramin and Pyramidon auf das Elektroencephalogramm (E.E.G.) und das Myelogramm von Katzen und Kaninchen. Dtsch Z Nervenheilk 164:395–406

Fifková E, Maršala J (1967) Stereotaxic atlases for the cat, rabbit and rat. In: Bureš J, Petrán M, Zachar J (eds) Electrophysiological methods in biological research. Academic Press, New York, pp 653–731

Gebhart GF (1982) Opiate and opioid peptide effects on brain stem neurons: relevance to nociception and antinociceptive pathways. Pain 12:93–140

Gebhart GF, Sandkühler J, Thalhammer JG, Zimmermann M (1983) Inhibition of spinal nociceptive information by stimulation in midbrain of the cat is blocked by lidocaine microinjected in nucleus raphe magnus and medullary reticular formation. J Neurophysiol 50:1446–1459

Jurna I (1963) Die Wirkung von 1-Phenyl-2,3-dimethyl-4-dimethylaminopyrazolon (Pyramidon) und 1-Phenyl-2,3-dimethyl-4-isopropylaminopyrazolon (Isopyrin) auf spinale Reflexe. Naunyn-Schmiedeberg's Arch Pharmacol 245:305–320

Jurna I (1972) Dämpfung repetitiver Aktivierungsvorgänge an der spinalen Motorik durch Morphin. In: Janzen R, Keidel WD, Herz A, Steichele C, Payne JP, Burt RA (eds) Schmerz (Pain). Thieme, Stuttgart, pp 267–269

Jurna I (1980) Effect of stimulation in the periaqueductal grey matter on activity in ascending axons of the rat spinal cord: selective inhibition of activity evoked by afferent Aδ and C fibre stimulation and failure of naloxone to reduce inhibition. Brain Res 196:33–42

Jurna I, Heinz G (1979) Differential effects of morphine and opioid analgesics on A and C fibre-evoked activity in ascending axons of the rat spinal cord. Brain Res 171:573–576

Jurna I, Heinz G, Blinn G, Nell T (1978) The effect of substantia nigra stimulation and morphine on α-motoneurones and the tail-flick response. Naunyn-Schmiedeberg's Arch Pharmacol 51:239–250

Jurna I, Zetler G (1981) Antinociceptive effect of centrally administered caerulein and cholecystokinin octapeptide (CCK-8). Eur J Pharmacol 73:323–331

Jurna I, Zetler G (1985) Effects on ascending nociceptive activity in the rat spinal cord produced by cholecystokinin octapeptide, ceruletide, and morphine injected into the periaqueductal gray matter. Ann NY Acad Sci 448:609–612

Lewis VA, Gebhart GF (1977) Evaluation of the periaqueductal central gray (PAG) as a morphine-specific locus of action and examination of morphine-induced and stimulation produced analgesia at coincident PAG loci. Brain Res 124:283–303

Mayer DJ, Wolfle H, Akil H, Carder B, Liebeskind JC (1971) Analgesia from electrical stimulation in the brain stem of the rat. Science 174:1351–1354

Pay S, Barasi S (1982) A study of the connections of nociceptive substantia nigra neurones. Pain 12:75–89

Reynolds DV (1969) Surgery in the rat during electrical analgesia induced by focal brain stimulation. Science 164:444–445

Starkenstein E, Hendrych F, Escobar-Bordoy J (1934) Zur experimentellen Analyse der Pyramidonwirkung. Naunyn-Schmiedeberg's Arch Pharmacol 176:486–493

Weithmann KU, Alpermann H-G (1985) Biochemical and pharmacological effects of dipyrone and its metabolites in model systems related to arachidonic acid cascade. Arzneimittelforsch 35:947–952

Wilcox GL, Carlsson K-H, Jochim A, Jurna I (1986) Mutual potentiation of antinociceptive effects of morphine and clonidine on motor and sensory responses in rat spinal cord. Brain Res (in press)

Yaksh TL, Rudy TA (1976a) Chronic catheterization of the spinal subarachnoid space. Physiol Behav 17:1031–1036

Yaksh TL, Rudy TA (1976b) Analgesia mediated by a direct spinal action of narcotics. Science 192:1357–1358

Yaksh TL, Rudy TA (1978) Narcotic analgesics: CNS sites and mechanisms of action as revealed by intracerebral injection techniques. Pain 4:299–359

Yeung JC, Rudy TA (1980) Multiplicative interaction between narcotic agonisms expressed at spinal and supraspinal sites of antinociceptive action as revealed by concurrent intrathecal and intracerebroventricular injections of morphine. J Pharmacol Exp Ther 215:633–642

Zieglgänsberger E (1985) Opioid actions on mammalian spinal neurones. Int Rev Neurobiol 25:243–275

Received August 13, 1986/Accepted October, 1986