Pharmacology Biochemistry and Behavior, Vol. 46, pp. 361–364, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Analgesic Effects of Antibiotics in Rats

CHARLES SUAUDEAU, ABDERRHAMAN CHAIT, CAROLINE CIMETIERE AND RENAUD DE BEAUREPAIRE[1]

INSERM U 320, Groupe de Psychopharmacologie Expérimentale, Laboratoire de Pharmacologie (Pr. Moulin), CHU Côte de Nacre, 14032 Caen, France

Received 3 August 1992

SUAUDEAU, C., A. CHAIT, C. CIMETIERE AND R. DE BEAUREPAIRE. *Analgesic effects of antibiotics in rats.* PHARMACOL BIOCHEM BEHAV 46(2) 361–364, 1993.—Studies in forelimb-deafferented rats suggest that treatment with certain antibiotics can decrease pain sensation. To test this hypothesis, the analgesic effects of nine randomly selected antibiotics were studied in rats by using a constant-temperature hotplate. The results show that several antibiotics have antinociceptive properties, and two of them, chloramphenicol and ampicillin, can produce analgesia in a dose range used in human therapy (100 mg/kg). This analgesia is comparable to salicylate and ketoprofen analgesia but lower than pethidine's one. The analgesia is long lasting with chloramphenicol (10 h or more). These antinociceptive properties cannot be attributed to sedation because amphetamine-induced hyperactivity, measured in an open field, is not sensitive to injection of the most sensitive antibiotics.

Pain Hotplate test Antibiotics Chloramphenicol Ampicillin

DAILY injections of certain antibiotics can suppress the self-mutilation behavior (autotomy) of rats with forelimb deafferentation after dorsal rhizotomy (9). It has been suggested that this autotomy is provoked by the pain animals feel in their deafferented limb (2,8). This interpretation is still controversial, and the literature for and against it can be found in several publications (1,9). It was thus important to determine whether the antibiotics are able by themselves to produce an analgesia by using tests traditionally employed in pharmacology. The putative analgesic properties of several randomly selected antibiotics were studied using an acute pain test, the latency to the first hindpaw lick on a constant-temperature hotplate. We compared the antibiotic actions with those of three analgesic drugs: an opiate compound, pethidine, and two antiinflammatory agents, salicylate and ketoprofen. To determine the latency and duration of the different drug effects, a time–response experiment was designed. Because the hotplate test cannot discriminate between a general sedative effect and a specific analgesic action, we also tested in an open field the hyperactivity provoked in animals by *d*-amphetamine after injections of the most effective antibiotics.

METHOD

Animals

The experiments were conducted on male Sprague–Dawley rats, weighing 250–350 g. Animals were housed in clear plastic cages in a room maintained in a 12 L : 12 D cycle (light 8:00 am–8:00 p.m.) and with free access to food and water.

Constant-Temperature Experiments

The day of the experiment, 5 h before the testing, animals were brought into the test room in their usual cages. They were SC injected with a drug solution or with the same amount of its solvent (controls) 45 min before the test. All experiments started at 2:00 p.m. The hotplate, a SOCREL DS-37, was set to give a constant plate temperature of 54 ± 0.5°C. Animals were placed on the hotplate, which was surrounded by a round, clear plastic cylinder (20 cm in diameter and 30 cm in height), and the latency of the first hindpaw lick was recorded. Experimentators were blind to the treatment of animals. Each animal was only used once.

Because no data was available from the literature for a protocol of testing for the analgesic effects of antibiotics, we performed a preliminary experiment using the two antibiotics previously found to be effective in preventing autotomy, namely, chloramphenicol and amoxicillin (9). We chose arbitrarily to test animals 45 min after the SC injection using four different dosages of the antibiotics: 30, 100, 300, and 600 mg/kg. This preliminary experiment showed that 45 min after injection of chloramphenicol an analgesic effect is observed with 100, 300, and 600 mg/kg but not with 30 mg/kg. Therefore, we used the 100- and 300-mg/kg dosages for further

[1] To whom requests for reprints should be addressed.

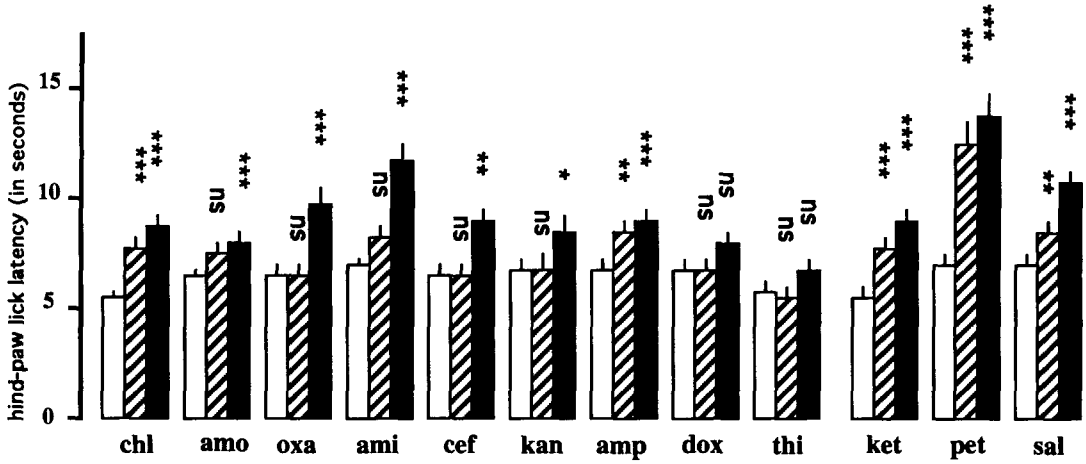

FIG. 1. Effects of antibiotics and analgesic drugs on pain sensation: Mean hindpaw lick latencies (±SEM). Open bars, controls; striped bars, 100 mg/kg; solid bars, 300 mg/kg. CHL, chloramphenicol; AMO, amoxicillin; OXA, oxacillin; AMI, amikacin; CEF, cefaprin; KAN, kanamycin; AMP, ampicillin; DOX, doxycycline; THI, thiamphenicol; KET, ketoprofen (striped bar, 1.3 mg/kg; solid bar, 4 mg/kg); PET, pethidine (striped bar, 1 mg/kg; solid bar, 5 mg/kg); SAL, salicylic acid. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

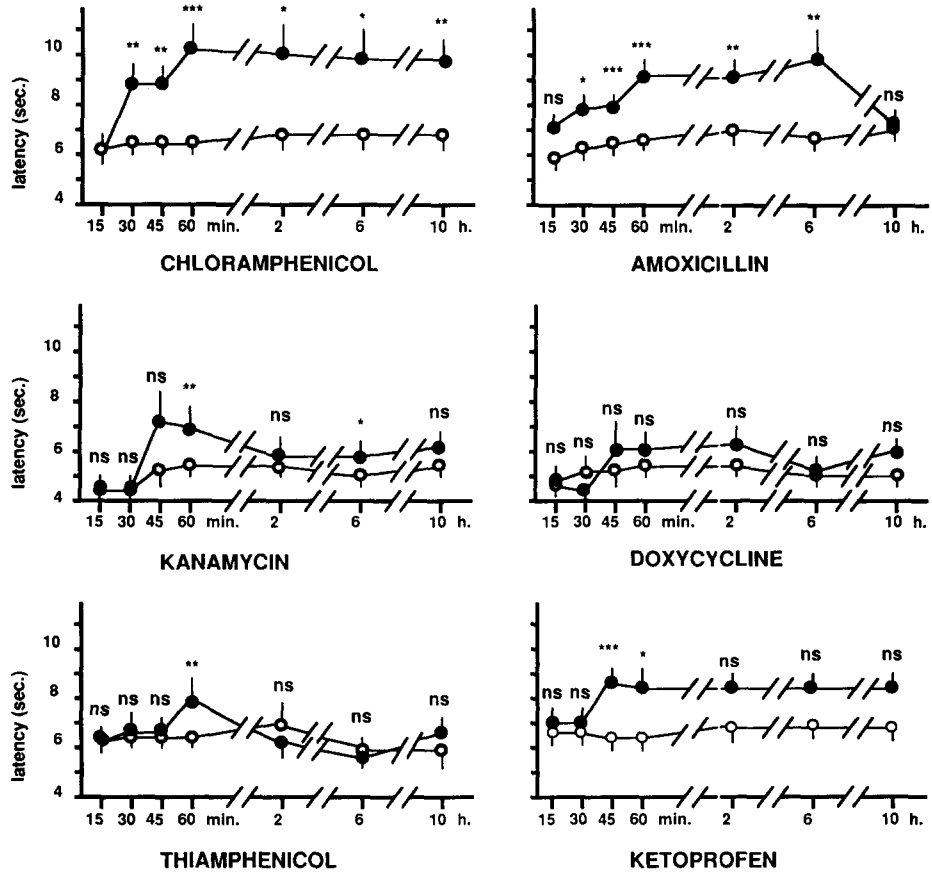

FIG. 2. Analgesic effect over time: Mean hindpaw lick latencies (±SEM). (○), controls; (●), treated. Hindpaw lick latencies (in seconds) are in ordinates. Times after injection (min or h) are in abscissa. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

experiments with other antibiotics and the 300-mg/kg dosage to study different periods of time after injection.

Drugs

The following compounds were tested: antibiotics—amoxicillin (Beecham Research Labs Ltd., Middlesex, UK), chloramphenicol (Synthelabo), thiamphenicol (Clin-Midy), doxycycline chlorhydrate (Pfizer, New York), amikacin, ampicillin, cefaprin, kanamycin, and oxacillin (Bristol-Myers, Syracuse, NY); antiinflammatory agents—salicylate (Synthelabo) and ketoprofen (Specia, Paris, France).

All drugs were buffered to obtain a pH solution between 6.5 and 7.5.

All antibiotics, as well as salicylate (100 and 300 mg/kg), were dissolved in distilled water (100 mg/ml). Ketoprofen was injected in the amount of 1.3 and 4 mg/kg using solutions of 1 mg per ml distilled water. Pethidine was injected in dosages of 1 and 5 mg/kg in 1 ml saline. Animals in the control groups (one control animal for one treated) were injected with the same amount of solvent. Eight animals were used for each dosage.

Time-Response Experiment

Seven different groups of animals were tested for seven periods of time after injection: 15, 30, and 45 min and 1, 2, 6, and 10 h. Five antibiotics were tested (300 mg/kg) and ketoprofen (4 mg/kg). Eight animals and eight controls were used for each period of time, and each animal was only used once.

Locomotor Activity Experiment

Possible changes in activity provoked by the antibiotics were searched for with the amphetamine-induced hyperactivity test. Three groups of 10 animals were used. In two of them, animals received an SC injection of one of the antibiotics that produced the clearest effect in the hotplate test (chloramphenicol and ampicillin, 300 mg/kg). Animals in the third group were injected with distilled water alone. Thirty minutes later, all animals received an IP injection of *d*-amphetamine (1.5 mg/kg). Immediately after this injection, each animal was placed alone in an open field (1 m^2 with lines on the floor) and locomotor activity was recorded for 50 min by counting the number of lines crossed by the animal every minute.

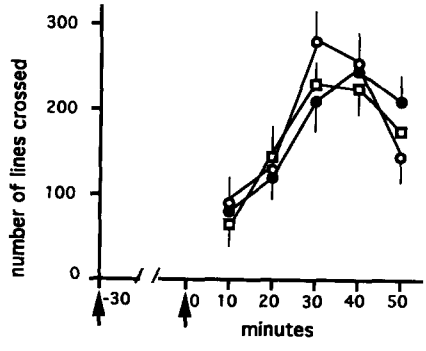

FIG. 3. Locomotor activity experiment (mean number of lines crossed) First arrow, injection time of antibiotics; second arrow, injection time of amphetamine. (○), controls; (●), chloramphenicol; (□), ampicillin. Some error bars have been omitted for clarity.

Statistical Analysis

The significance of the different results was evaluated using Student's *t*-test for unmatched pairs.

RESULTS

The results show that with the lowest dosage (100 mg/kg) only chloramphenicol and ampicillin have a significant analgesic effect; the changes in hindpaw lick latency are not significant for the other antibiotics (Fig. 1). With the 300-mg/kg treatment, chloramphenicol, amoxicillin, ampicillin, oxacillin, amikacin, and cefaprin significantly increase the lick latency. The analgesia observed is comparable to that produced by salicylate (300 mg/kg) and ketoprofen (1.3 and 4 mg/kg) and always weaker than with pethidine. Doxycycline and thiamphenicol have no significant effect with any dosage.

The time-response effects of five antibiotics with the 300-mg/kg dosage and of ketoprofen (4 mg/kg) are presented in Fig. 2. Two antibiotics (kanamycin and thiamphenicol) that have no analgesic properties 45 min after injection are found to be analgesic 1 h after injection. Analgesia duration is variable depending upon the antibiotic. The longest effect is produced by chloramphenicol, starting 30 min after injection and still present 10 h later. The analgesic effect of amoxicillin starts 15 min after injection, lasts 6 h, and disappears between 6 and 10 h. The effect of ketoprofen is shorter, only significant 45 min and 1 h after injection.

Chloramphenicol and ampicillin, the most active antibiotics on the hotplate test, have no effects on amphetamine-induced hyperactivity (Fig. 3). Consequently, the analgesic effects of the antibiotics are presumably not related to a general sedative effect.

DISCUSSION

Among nine antibiotics randomly selected, eight have a significant analgesic effect on the hotplate test. The most effective is chloramphenicol, whose analgesic properties are comparable to those of salicylate and ketoprofen for all dosages tested, with a length of action exceeding 10 h. Amoxicillin, amikacin, and oxacillin also have strong analgesic effects but only with the higher dosages. Doxycycline effects do not significantly differ from the solvent in the conditions used. Kanamycin and thiamphenicol have only a weak and short effect.

As far as we know, such analgesic properties have never been ascribed before to antibiotics, although the possibility of an analgesic action of chloramphenicol associated with triamcinolone in postoperative pain has been raised by Lin and Lan (7).

These analgesic properties do not depend upon a particular class of antibiotics. Two phenicolated compounds were tested and one, chloramphenicol, was the most active of the nine tested while the other, thiamphenicol, was poorly active. Among the aminosides, amikacin showed a strong analgesic effect and kanamycin a weak one. All penicillins tested (oxacillin, amoxicillin, and ampicillin) had analgesic effects but with different dosages and duration of action. The analgesia does not seem to be related to the capacity to cross the blood-brain barrier because chloramphenicol and thiamphenicol cross it similarly with different analgesic effects.

The dosages used in these experiments were generally higher than the usual therapeutic ones given to humans. But, for two antibiotics, chloramphenicol and ampicillin, the lowest dose tested (100 mg/kg) was within the range of the highest

therapeutic dosages recommended for humans. However, it is difficult to compare dosages between rats and humans because of susceptibility differences between species.

It is interesting to note that chloramphenicol and amoxicillin, which are the most active antibiotics for the suppression of autotomy behavior in deafferented animals (9), have a significant analgesic effect on the hotplate experiment. This finding favours the hypothesis that autotomy is provoked by a pain referred to the forelimb after deafferentation.

It is now important to determine the mechanism by which antibiotics have such analgesic properties. Some effects other than antiinfectious have already been attributed to them, such as allergic reactions or electroencephalographic abnormalities (6). Certain antibiotics are neuromuscular blockers (5) and this effect has been related to a depressive action on calcium currents (4). Antibiotics may as well have an analgesic effect by blocking an activity related to pain sensation at some central level that remains to be determined. However, penicillins cross the blood-brain barrier poorly and have significant analgesic effects. Moreover, in recent unpublished results we have shown that intracerebral injections [300 μg intraventricularly and 30 μg in brain sites specifically involved in the central analgesic effects of pharmacological agents (3)] of various penicillins do not change pain sensitivity. Therefore, the analgesic effects of antibiotics seem to us more likely to be peripheral than central. Another possible interpretation of our results is that administration of relatively high doses of antibiotics in rats is producing a stress effect responsible for the analgesic effect. We cannot definitely exclude this eventuality. However, such a stress effect is unlikely because stress also produces important changes in locomotor activity (hyper- or hypoactivity) and we found no change in activity in antibiotic-treated animals. Moreover, stress is centrally mediated, and if the antibiotic-induced analgesia was a stress effect one would expect intracerebral injections of antibiotics to have altered the pain sensitivity of animals. In conclusion, our hypothesis is that antibiotic analgesia is a peripheral effect.

The knowledge that some antibiotics have analgesic properties could be useful in the choice of an antibiotic for the treatment of patients with deafferentation pain and also of certain pathologic conditions in which an infection is associated with a painful syndrome, for example, meningitis or dental infection.

ACKNOWLEDGEMENTS

Drugs were kindly provided by Beecham, Bristol-Myers, Clin-Midy, Pfizer, Specia, and Synthelabo.

REFERENCES

1. Albe-Fessard, D.; Giambernardino, M. A.; Rampin, O. Comparison between different animal models of chronic pain. In: Lipton, S., ed. Advances in pain research and therapy. vol. 12. New York: Raven Press; 1989:11-27.
2. Basbaum, A. Effects of central lesions on disorders produced by multiple dorsal rhizotomy in rats. Exp. Neurol. 42:490-501; 1974.
3. de Beaurepaire, R.; Suaudeau, C.; Chait, A.; Cimetière, C. Anatomical mapping of brain sites involved in the antinociceptive effects of ketoprofen. Brain Res. 536:201-206; 1990.
4. Bourret, C.; Mallart, A. Depression of calcium currents at mouse motor nerve endings by polycationic antibiotics. Brain Res. 478: 403-406; 1989.
5. Caputy, A. J.; Kim, Y. I.; Sanders, D. B. The neuromuscular blocking effects of therapeutic concentrations of various antibiotics on normal rat skeletal muscles: A quantitative comparison. J. Pharmacol. Exp. Ther. 217:369-377; 1981.
6. Doublik, S.; Vacek, V.; Vacek, Z. Electroencephalic changes of antibiotics. Arzneimittel-Forsch. 17:1177-1181; 1967.
7. Lin, C. P.; Lan, W. H. Intracanal application with triamcinolone-chloramphenicol on postoperative pain after immediate root canal filling. J. Formosan Med. Assoc. 84:13368-13372; 1985.
8. Lombard, M. C.; Nashold, B. S.; Albe-Fessard, D. Deafferentation hypersensitivity in the rat after dorsal rhizotomy: A possible animal model of chronic pain. Pain 6:163-174; 1979.
9. Suaudeau, C.; de Beaurepaire, R.; Rampin, O.; Albe-Fessard, D. Antibiotic and morphinomimetic injections prevent automutilation behavior in rats after dorsal rhizotomy. Clin. J. Pain 5:177-181; 1989.