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The narcotic discriminative stimulus complex: relation to analgesic activity

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The ability of drugs to produce the narcotic discriminative stimulus complex is found to be highly correlated with their analgesic activity; in contrast, no relation with their antidiarrhoeal activity is evident. The findings suggest that the narcotic discriminative stimulus complex is a centrally mediated effect of narcotic drugs.

The narcotic analgesics fentanyl (Janssen, Niemegeers & Dony, 1963) and morphine have been shown (Colpaert, Lal & others, 1975a; Gianutsos & Lal, 1975; Hill, Jones & Bell, 1971; Hirschhorn & Rosecrans, 1974; Rosecrans, Goodloe & others, 1973) to produce a discriminative stimulus complex in rats. That is, rats can be trained to make one response when being treated with a narcotic analgesic and to emit another response upon solvent injection. The stimulus complex produced by fentanyl and other narcotic analgesics has been defined (Colpaert & others, 1975a) as the discriminative stimulus complex exclusively associated with the specific central actions produced by narcotic drugs.

The present study sought to investigate whether the ability of drugs to produce the narcotic discriminative stimulus complex would be related to their potency with respect to other characteristic actions of narcotic drugs. Any such relation would undoubtedly contribute to the identification of the pharmacological activity underlying the narcotic discriminative stimulus complex. The two alternative actions with which the potency to produce the narcotic discriminative stimulus complex was compared, are narcotic drug induced analgesic and antidiarrhoeal effects; these actions are considered to represent central (Ayhan, 1972; Cicero, 1974; Jacquet & Lajtha, 1973; Johannesson, 1967; Reinhold, Bläsig & Herz, 1973) and peripheral (Janssen & Jageneau, 1957; Niemegeers, Lenaerts & Awouters, 1975; Türker & Kaymakçalan, 1971; Van Nueten, Janssen & Fontaine, 1974) activity of narcotic analgesic drugs respectively. To this end, six typical narcotic analgesics and three typical antidiarrhoeal drugs were comparatively investigated for analgesic and antidiarrhoeal activity, as well as for their potency to produce the narcotic discriminative stimulus complex.

METHODS

Drug discrimination

The ability of drugs to produce the narcotic discriminative stimulus complex was investigated by a procedure described elsewhere (Colpaert & others, 1975a; Colpaert & Niemegeers, 1975). In short, rats were trained to discriminate fentanyl (1·25 mg kg^{-1}, orally) from solvent (orally). Discrimination was evidenced by the rats pressing (fixed ratio 10) either of two levers in order to obtain food. That is, 60 min following fentanyl or solvent administration, the animals were allowed to press either the

fentanyl lever or the solvent lever; reinforcement was only available upon pressing the lever appropriate to the treatment. After two months of training, the rats were perfectly trained to always select the appropriate lever. Once this training level was reached, test drugs were given (orally) 60 min before testing, and lever selection following various test treatments was assessed. Fentanyl lever selection following test treatment indicates that the animal generalizes the test treatment to the standard fentanyl treatment, and thus reveals the test drug to produce the narcotic discriminative stimulus complex (all-or-none criterion).

At least five thus trained animals were used per dose of each test drug; different doses of each drug were given so as to enable the computation of ED50 values (Litchfield & Wilcoxon, 1949).

Analgesia

Experimentally naive male Wistar rats, 200 $\pm$ 10 g, starved overnight were used once only.

The procedure used to assess analgesic drug action measures the inhibitory effects of drugs on the Tail Withdrawal Reflex, and has been extensively described (Janssen & others, 1963). In short, solvent (controls) or drug was orally administered by gavage, and immediately thereafter the animals were placed in a standard rat holder with the tail hanging freely outside. Sixty min later, the tail was dipped into a warm (55 $\pm$ 1°) water bath. The reaction time was defined as the time elapsing between the moment the tail was dipped into the water and the moment it was withdrawn by the rat.

Five to 20 animals were used per dose of each drug.

Large-scale control data obtained with this procedure (Janssen & others, 1963; Janssen, Niemegeers & others, 1971) showed that only 2·4% (out of 600 solvent treated rats) of the control animals had a reaction time $\geqslant$6 s. Therefore, a drug-treated rat is considered to be affected by the analgesic effect of the drug if its reaction time exceeds 6 s. According to the latter criterion, ED50 values were computed using the method of Litchfield & Wilcoxon (1949).

Antidiarrhoeal action

The animals used were similar to those used in the previous procedure; they too were used once only.

The procedure used to assess constipating drug action measures the protecting effects of drugs on castor oil-induced diarrhoea, and has been described (Niemegeers, Lenaerts & Janssen, 1974a, b). Rats were pretreated with either solvent (controls) or drug; 1 h later, each animal was challenged with an oral dosage of 1 ml castor oil.

Large-scale experiments (Niemegeers & others, 1975) demonstrated that 95·4% (out of 1000 solvent-pretreated rats) of the control animals show profuse diarrhoea within 1 h after the castor oil administration. Therefore, the absence of diarrhoea within 1 h after castor oil dosage was considered as the criterion for a protecting drug effect, and ED50 values (Litchfield & Wilcoxon, 1949) were calculated according to this criterion. Five to 10 animals were used per dose of each drug.

Drugs and doses

The doses used were selected from the geometrical series 0·01, 0·02, . . ., 80, 160, and were expressed in mg kg^{-1}.

All drugs and solvent were administered at a constant volume of 1 ml per 100 g body weight and were freshly prepared immediately before use. Fentanyl citrate was dissolved in a solvent containing 9 mg NaCl, 0·5 mg methylparasept and 0·05 mg propylparasept ml^{-1}. Bezitramide HCl was used as an aqueous suspension containing 1% polysorbate 80. To the loperamide HCl concentrations of 2 mg ml^{-1} and higher, 10% propylene glycol was added. At the concentration of 2 mg ml^{-1}, diphenoxylate HCl and difenoxin HCl were given in aqueous suspensions, micronized with an ultrasonic sonifier. Codeine phosphate, dextromoramide tartrate, methadone HCl and morphine HCl were dissolved in water. The doses of fentanyl and dextromoramide refer to the base; the doses of the other drugs refer to the salt.

RESULTS

The data obtained with all three procedures are summarized in Table 1.

Table 1. *ED50 values (and 95% confidence limits) of different drugs with respect to their ability to produce the narcotic discriminative stimulus complex (drug discrimination), to produce analgesia (tail withdrawal in rats) and to antagonize diarrhoea (castor oil test) in rats.*

	Narcotic discriminative stimulus complex ED50 (mg kg^{-1})	Analgesia ED50 (mg kg^{-1})	Antidiarrhoea ED50 (mg kg^{-1})	Stimulus-ED50 / Analgesia-ED50	Analgesia-ED50 / Antidiarrhoea-ED50
Dextromoramide	1·78 (1·31–2·42)	1·98 (1·52–2·57)	1·80 (1·13–2·86)	0·90	1·10
Bezitramide	0·57 (0·40–0·82)	0·38 (0·25–0·58)	0·26 (0·16–0·41)	1·50	1·46
Fentanyl	0·63 (0·51–0·78)	0·42 (0·28–0·64)	0·19 (0·12–0·31)	1·50	2·21
Methadone	8·50 (5·86–12·3)	11·6 (8·16–16·5)	2·19 (1·50–3·21)	0·73	5·30
Codeine	22·7 (15·9–32·5)	19·0 (13·3–27·7)	2·85 (1·87–4·35)	1·19	6·67
Morphine	20·0 (13·0–30·8)	13·5 (8·58–21·2)	1·52 (1·01–2·27)	1·48	8·88
Diphenoxylate	7·10 (4·90–10·3)	6·93 (3·34–14·4)	0·15 (0·11–0·22)	1·02	46·2
Difenoxin	2·50 (1·77–3·54)	2·57 (1·57–4·21)	0·04 (0·03–0·07)	0·97	64·2
Loperamide	>40	⩾160*	0·15 (0·11–0·20)	—	>1,067

All drugs were orally administered 60 min before the assessment of its stimulus properties and of analgesia, and 60 min before castor oil treatment.
*Higher doses could not be tested; the oral LD50 of loperamide is: 184 (135–254) mg kg^{-1}.

All compounds were fully active in all three procedures and appropriate ED50 values could be established. Loperamide, however, only antagonized castor oil-induced diarrhoea. Testing of higher doses of this compound was impaired by toxicity (at doses higher than 160 mg kg^{-1}) in the analgesia procedure and by complete suppression of responding (at 80 mg kg^{-1}) in the drug discrimination procedure.

It is apparent that all the ED50 values for narcotic discriminative stimulus complex fall within the 95% confidence range of the analgesia-ED50's. Correspondingly, the values of the stimulus-ED50 : analgesia-ED50 ratio range only between 0·73 and 1·50, indicating that little difference in the absolute doses was required to produce the narcotic discriminative stimulus complex on the one hand, and analgesia on the other. Computation of the Spearman Rank Correlation Coefficient (Siegel, 1956) reveals a perfect ($rs = +1·0$) correlation between stimulus properties and analgesic activity ($P<0·01$, one-tailed).

In contrast, the stimulus-ED50 values largely exceed the antidiarrhoea-ED50's (except for dextromoramide). The analgesia-ED50 : antidiarrhoea-ED50 ratio reveals very marked differences between compounds with respect to their relative ability to produce analgesia and to antagonize castor oil-induced diarrhoea. No

relation is evident ($rs = +0{\cdot}17$; $P > 0{\cdot}05$) either between stimulus properties and antidiarrhoeal activity, or between analgesic and antidiarrhoeal activity.

DISCUSSION

The data reported herein indicate that there exists an extremely high positive correlation ($rs = 1{\cdot}0$) between the analgesic effects of drugs and their ability to produce the narcotic discriminative stimulus complex in rats. This finding strongly suggests that both effects of narcotic drugs are subserved either by an identical pharmacological action or by two distinct actions with similar dose-effect characteristics. The first alternative would seem to be consistent with the observation that the stimulus-ED50 : analgesia-ED50 ratio varies within the close vicinity of one. No correlation was evident between the stimulus properties and the anti-diarrhoeal activity of drugs; both effects were also clearly dissociated in terms of absolute doses. The analgesia-ED50 : antidiarrhoea-ED50 ratio (and, similarly, the stimulus-ED50 : antidiarrhoea-ED50 ratio) indicates very marked differences between drugs with regard to their relative central and peripheral effects. Thus, for example, dextromoramide produces its central and peripheral effects at essentially the same dose (1·80 to 1·98 mg kg^{-1}, orally); the other five narcotics are ranked according to increasing ratio (Table 1), with morphine possessing the largest dissociation (i.e. 8·88). The antidiarrhoeals diphenoxylate and difenoxin are typically characterized by an outstanding dissociation between analgesic and antidiarrhoeal activity (ratio 46·2 and 64·2), whereas no central effect is evident following loperamide at doses which exceed its antidiarrhoea-ED50 with a factor 1067 or more. These findings, then, corroborate the contention (Colpaert, Niemegeers & others, 1975b; Gianutsos & Lal, 1975) that the antidiarrhoeal effects of narcotic drugs are not responsible for (and independent from) their stimulus properties in rats. In fact, the finding that the stimulus properties of those drugs are highly correlated with a central, but not with a peripheral effect of the same drugs, suggests that the narcotic discriminative stimulus complex is of central origin (Colpaert & others, 1975a).

Apart from the mere fact that narcotic analgesics produce a discriminative stimulus complex in rats (Colpaert & others, 1975a; Gianutsos & Lal, 1975; Hill & others, 1971), very little is known on the mechanism underlying this action. The finding that naloxone (0·2 to 0·8 mg kg^{-1}, i.p.) and *p*-chlorophenylalanine (350 mg kg^{-1}, orally) antagonize the stimulus properties of morphine (20 mg kg^{-1}, i.p.), whereas α -methyl-*p*-tyrosine (135 mg kg^{-1}, i.p.) does not (Rosecrans & others, 1973) points to a role for 5-hydroxytryptamine in this stimulus complex. Also, tolerance to the stimulus effects of morphine has recently been reported (Hirschhorn & Rosecrans, 1974). Similarly, 5-hydroxytryptamine is considered to be involved in the analgesic action of narcotic drugs (Calcutt, Handley & others, 1972), whereas the development of tolerance to narcotic drug-induced analgesia is a well known phenomenon.

The data thus far available are consistent with the hypothesis that the stimulus properties and analgesic actions of narcotic drugs are subserved by very similar, if not by an identical central mechanism.

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REFERENCES

AYHAN, I. H. (1972). *Psychopharmacologia*, **25**, 183–188.

CALCUTT, C. R., HANDLEY, S. H., SPARKES, C. G. & SPENCER, P. S. J. (1972). In: *Agonist and antagonist actions of narcotic analgesic drugs*, pp. 176–191. Editors; Kosterlitz, H. W., Collier, H. O. J. & Villareal, J. E. London: MacMillan.

CICERO, T. J. (1974). *Archs int. Pharmacodyn, Thér.*, **208**, 5–13.

COLPAERT, F. C., LAL, H., NIEMEGEERS, C. J. E. & JANSSEN, P. A. J. (1975a). *Life Sci.*, **16**, 705–716.

COLPAERT, F. C., NIEMEGEERS, C. J. E., LAL, H. & JANSSEN, P. A. J. (1975b). *Ibid.*, **16**, 717–728.

COLPAERT, F. C. & NIEMEGEERS, C. J. E. (1975). *Archs int. Pharmacodyn. Thér.*, **217**, 170–172.

GIANUTSOS, G. & LAL, H. (1975). *Psychopharmacologia*, **41**, 267–270.

HILL, H. E., JONES, B. E. & BELL, E. C. (1971). *Ibid.*, **22**, 305–313.

HIRSCHHORN, I. D. & ROSECRANS, J. A. (1974). *Ibid.*, **36**, 243–253.

JACQUET, Y. F. & LAJTHA, A. (1973). *Science*, **182**, 490–492.

JANSSEN, P. A. J. & JAGENEAU, A. H. (1957). *J. Pharm. Pharmac.*, **9**, 381–400.

JANSSEN, P. A. J., NIEMEGEERS, C. J. E. & DONY, J. G. H. (1963). *Arzneimittel-Forsch.*, **13**, 502–507.

JANSSEN, P. A. J., NIEMEGEERS, C. J. E., SCHELLEKENS, K. H. L., MARSBOOM, R. H. M., HÉRIN, V. V. & AMERY, W. K. P. (1971). *Ibid.*, **21**, 862–867.

JOHANNESSON, T. (1967). *Acta pharmac. tox.*, **25**, *Suppl.* 3, 1–83.

LITCHFIELD, J. T. & WILCOXON, F. (1949). *J. Pharmac. exp. Ther.*, **96**, 99–113.

NIEMEGEERS, C. J. E., LENAERTS, F. & AWOUTERS, F. (1975). In: *Synthetic antidiarrheal drugs*. Editors: Van Bever, W. & Lal, H. N.Y.: Dekker M., Inc., in the press.

NIEMEGEERS, C. J. E., LENAERTS, F. M. & JANSSEN, P. A. J. (1974a). *Arzneimittel-Forsch.*, **24**, 1633–1636.

NIEMEGEERS, C. J. E., LENAERTS, F. M. & JANSSEN, P. A. J. (1974b). *Ibid.*, **24**, 1636–1641.

REINHOLD, K., BLÄSIG, J. & HERZ, A. (1973). *Arch. exp. Path. Pharmak.*, **278**, 69–80.

ROSECRANS, J. A., GOODLOE, M. H., BENNETT, G. J. & HIRSCHHORN, I. D. (1973). *Eur. J. pharmac.*, **21**, 252–256.

SIEGEL, S. (1956). *Nonparametric statistics for the behavioural sciences*, pp. 312. McGraw-Hill: New York.

TÜRKER, R. K. & KAYMAKÇALAN, S. (1971). *Archs int. Pharmacodyn. Thér.*, **193**, 397–404.

VAN NUETEN, J. M., JANSSEN, P. A. J. & FONTAINE, J. (1974). *Arzneimittel-Forsch.*, **24**, 1641–1645.