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# INTERACTIONS OF PENTAZOCINE AND NALOXONE ON THE MONOAMINE CONTENT OF DISCRETE REGIONS OF THE RAT BRAIN*

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**Abstract**—Pentazocine, 5·6 to 56 mg/kg, caused dose-related decreases in the catecholamine content of discrete regions of the rat brain. Norepinephrine levels were lowered in all brain regions examined, whereas the depletion of dopamine was restricted to the cortex and striatum. Serotonin levels were relatively unaffected by pentazocine. Pretreatment with naloxone antagonized the pentazocine-induced depletion of brain dopamine. Naloxone also blocked the pentazocine-induced depletion of norepinephrine in the cortex and midbrain, but failed to affect the depletion of norepinephrine in the medulla and actually enhanced the depletion of hypothalamic norepinephrine. A significant depletion of medullary serotonin was observed only in rats pretreated with naloxone. These findings are consistent with the concept that pentazocine's agonistic spectrum of activity involves two components: one which is blocked by naloxone and one which is not.

THE FINDING that morphine and related drugs can deplete the brain of norepinephrine (NE) in a variety of species[1–4] has stimulated numerous investigations of the role of this neurohumor in some of the actions of the narcotic analgesics. Two other putative neurotransmitters, dopamine (DA) and serotonin (5-hydroxytryptamine, 5-HT), have also been implicated in the actions of morphine. Although morphine does not consistently reduce the brain content of DA and 5-HT, there is evidence that the rate of synthesis of DA and 5-HT is increased after the acute or chronic administration of morphine.[5]

It is now becoming apparent that narcotic-antagonist analgesics can also affect the disposition of brain monoamines. One such drug, pentazocine, lowers the total brain content of both NE and DA in the rat, but has only a slight effect on the concentration of 5-HT when tested over a dose range of 8·0 to 64 mg/kg.[6] The depletion of brain catecholamines by pentazocine is closely associated with stimulation of locomotor activity.[6] Naloxone, a potent narcotic antagonist that is virtually devoid of agonistic activity,[7,8] blocks neither the depletion of brain monoamines nor the stimulation of locomotor activity produced by pentazocine. This is unusual insofar as most of pentazocine's agonistic actions (for example, analgesia and respiratory depression) are antagonized by naloxone.[9,10] The failure of naloxone to block some of the effects of pentazocine suggests that pentazocine's agonistic component of action is mediated by at least two distinct mechanisms: one which is blocked by naloxone and one which is not.

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In our earlier study,[6] the effects of pentazocine and naloxone were examined on the monoamine content of the whole brain. Thus, it was not possible to ascertain whether the depletion of catecholamines by pentazocine was localized or was occurring throughout the brain. Similarly, an antagonism of pentazocine's effects in a discrete area of the brain by naloxone could easily have been masked by changes occurring on other brain areas. Hence, the purpose of the present study was to determine the effects of pentazocine on the NE, DA and 5-HT content of discrete regions of the rat brain. Pentazocine was tested alone and after pretreatment with naloxone.

## METHODS

*Subjects.* The subjects were male CFE rats (Carworth, Division of Becton, Dickinson & Co., New City, N.Y.) weighing 175–250 g. The rats were housed in group cages in a large colony room for at least 1 week prior to being used in the study. The colony room was artificially illuminated between 6:00 a.m. and 6:00 p.m.

*Dissection of the brain.* The rats were killed by decapitation and the brain was rapidly removed and placed on a cold plate maintained at approximately −5·0 to −10°. The following five regions of the brain were then removed using essentially the guidelines of Glowinski and Iverson:[11] cortex, striatum, hypothalamus, midbrain, medulla. Table 1 shows the average wet weight of each of the brain regions. Homogenates were prepared by sonication of the brain sections in 5 or 10 ml of ice-cold acid–butanol containing ethylenediamine tetraacetic acid and metabisulfite. In some experiments the whole brain minus the cerebellum was homogenized in 20 ml of acid–butanol. The content of NE, DA and 5-HT in the brain homogenates was determined fluorometrically by the method of Ansell and Beeson,[12] with a modification[13] to permit developing the DA fluorophore on the same day that the assay was performed rather than on the following day. At least 25 per cent of the samples in a given assay were taken from control (i.e. vehicle-treated) animals. Internal standards were also included in every assay. The mean (±1 S. E. M.) recoveries of NE, DA and 5-HT were 83·6 ± 1·8, 43·2 ± 1·2 and 64·4 ± 2·9 per cent respectively.

*Drugs.* The drugs used in this study were naloxone hydrochloride and pentazocine as the free base. Naloxone was dissolved in 0·9% saline. Pentazocine was dissolved in three parts of 8·5% lactic acid; two parts of 1·0 N sodium hydroxide were then added to give the final solution a pH of between 4 and 5. Drugs were injected sub-

TABLE 1. WEIGHT OF DISCRETE REGIONS OF RAT BRAIN

| Brain region | Weight (mg) |
|---|---|
| Cortex | 813 ± 46* |
| Striatum | 70 ± 7 |
| Hypothalamus | 110 ± 12 |
| Midbrain | 216 ± 27 |
| Medulla | 243 ± 22 |

* Each value represents the mean ± 1 S.D. of 60 samples.

cutaneously in a volume of 1·0 ml/kg of body weight. Since it had previously been demonstrated that monoamine levels in the whole brain exhibit the greatest change 1 hr after the administration of pentazocine,[6] dose–response curves for the drug were determined using this time interval. Pentazocine (5·6, 17·5 and 56 mg/kg) or pentazocine vehicle was injected 60 min before the rats were killed. Naloxone (10 mg/kg) or saline was injected 5 min earlier. This dose of naloxone was selected because it is effective in blocking the actions of pentazocine on continuous avoidance responding in the rat.[6]

*Analysis of data.* The treatment means for a given brain region were compared to the mean of the control values for that region with a two-sided Dunnett's test.[14] In addition, for each brain region, the effects of the three doses of pentazocine alone were compared to those of the three doses of pentazocine following naloxone pretreatment by analysis of variance with partitioning the treatment sum of squares for main effect.[15] A treatment effect was considered to be significant if the resulting P value was less than 0·05.

## RESULTS

The effects of pentazocine alone and after pretreatment with naloxone on monoamine levels in the various brain regions and in the whole brain are presented in Tables 2–4. The concentrations of NE and 5-HT in the striatum and of DA in the hypothalamus were at the lower limits of the sensitivity of the assay and could not be reliably measured. Consequently, those data are not included in the tables.

Pentazocine had the broadest effect on brain NE. The levels of this neurohumor were reduced in all of the brain regions examined as well as in whole brain (Table 2).

TABLE 2. EFFECTS OF PENTAZOCINE AND NALOXONE ON NOREPINEPHRINE CONTENT OF DISCRETE REGIONS OF RAT BRAIN*

| Dose (mg/kg) | | Regional NE content (ng/g of tissue) | | | | |
|---|---|---|---|---|---|---|
| Naloxone | Pentazocine | Cortex | Hypothalamus | Midbrain | Medulla | Whole brain |
| 0 | 0 | 483 ± 27 | 1373 ± 49 | 418 ± 17 | 729 ± 26 | 465 ± 15 |
| 0 | 5·6 | 373 ± 23 | 1303 ± 127 | 393 ± 30 | 764 ± 70 | 417 ± 14 |
| 0 | 17·5 | 333 ± 20‡ | 1189 ± 81‡ | 310 ± 27‡ | 677 ± 39 | 389 ± 11† |
| 0 | 56 | 284 ± 19‡ | 1106 ± 97‡ | 256 ± 10‡ | 567 ± 37† | 339 ± 22‡ |
| 10 | 0 | 495 ± 42 | 1434 ± 130 | 331 ± 19‡ | 659 ± 30 | 449 ± 14 |
| 10 | 5·6 | 477 ± 27 | 1114 ± 82† | 400 ± 9 | 687 ± 30 | 461 ± 12 |
| 10 | 17·5 | 488 ± 24 | 1037 ± 72† | 385 ± 28 | 693 ± 28 | 417 ± 21 |
| 10 | 56 | 426 ± 28 | 1001 ± 62† | 319 ± 21‡ | 569 ± 14† | 359 ± 19‡ |
| P value, pentazocine (5·6, 17·5, 56 mg/kg) vs naloxone + pentazocine | | 0·001 | 0·05 | 0·025 | NS | NS |

* Rats were killed 65 min after s.c. administration of naloxone and 60 min after pentazocine. The values presented for the whole brain are exclusive of the cerebellum. Each value represents a mean ± 1 S. E. M. of at least six observations. NS = not significant.

† Significantly different from vehicle-treated controls (top line), $P < 0.05$.

‡ Significantly different from vehicle-treated controls (top line), $P < 0.01$.

TABLE 3. EFFECTS OF PENTAZOCINE AND NALOXONE ON DOPAMINE CONTENT OF DISCRETE REGIONS OF RAT BRAIN*

| Dose (mg/kg) | | Regional DA content (ng/g of tissue) | | | | |
|---|---|---|---|---|---|---|
| Naloxone | Pentazocine | Cortex | Striatum | Midbrain | Medulla | Whole brain |
| 0 | 0 | 969 ± 37 | 16,782 ± 386 | 241 ± 27 | 177 ± 24 | 1352 ± 35 |
| 0 | 5·6 | 768 ± 45† | 14,064 ± 1,176‡ | 211 ± 50 | 250 ± 46 | 1276 ± 37 |
| 0 | 17·5 | 718 ± 25† | 14,258 ± 686‡ | 205 ± 61 | 217 ± 43 | 1091 ± 35‡ |
| 0 | 56 | 542 ± 10‡ | 11,792 ± 981‡ | 194 ± 32 | 140 ± 16 | 873 ± 39‡ |
| 10 | 0 | 956 ± 83 | 17,349 ± 1025 | 206 ± 39 | 196 ± 55 | 1306 ± 25 |
| 10 | 5·6 | 984 ± 112 | 16,220 ± 771 | 263 ± 45 | 185 ± 44 | 1348 ± 75 |
| 10 | 17·5 | 912 ± 93 | 16,036 ± 520 | 205 ± 31 | 154 ± 43 | 1219 ± 48 |
| 10 | 56 | 725 ± 69† | 12,692 ± 371‡ | 225 ± 27 | 143 ± 6 | 1013 ± 76‡ |
| P value, pentazocine (5·6, 17·5, 56 mg/kg) vs naloxone + pentazocine | | 0·005 | 0·025 | NS | NS | 0·05 |

* Rats were killed 65 min after s.c. administration of naloxone and 60 min after pentazocine. The values presented for the whole brain are exclusive of the cerebellum. Each value represents a mean ± 1 S. E. M. of at least six observations. NS = not significant.

† Significantly different from vehicle-treated controls (top line), $P < 0.05$.

‡ Significantly different from vehicle-treated controls (top line), $P < 0.01$.

The cortex and midbrain were the areas most sensitive to the NE-depleting effect of pentazocine. NE levels were reduced by as much as 41·2 and 38·8 per cent in the cortex and midbrain respectively; this is about twice the depletion observed in the hypothalamus and medulla.

Brain DA appeared to be even more sensitive to depletion by pentazocine than was brain NE. Significant reductions in the DA content of the cortex and striatum were produced by as little as 5·6 mg/kg of pentazocine (Table 3). The effects of pentazocine on brain DA levels were limited to the cortex and striatum, in contrast to the more widespread effects that this drug exerted on the brain levels of NE. The cortex and striatum were also the regions that contained the most substantial amounts of DA.

5-HT concentrations were relatively unaffected by the administration of pentazocine. Although downward trends in 5-HT content were noted in most regions of the brain, a significant reduction in 5-HT content (40·6 per cent, $P < 0.01$) occurred only in the midbrain after treatment with 56 mg/kg of pentazocine. Not surprisingly, this limited effect of pentazocine was not reflected in the values of 5-HT obtained for the whole brain.

Naloxone (10 mg/kg) injected alone 65 min before decapitating the rats had, with one exception, no effect on the brain content of any of the three monoamines. Naloxone did produce a significant reduction (20·8 per cent, $P < 0.01$) of NE concentration in the midbrain (Table 2).

When naloxone was administered 5 min before pentazocine, the resulting effects ranged from significant antagonism to significant enhancement of the pentazocine-induced depletion of the brain monoamines. Naloxone completely prevented the depletion of NE in the cortex produced by pentazocine, and significantly attenuated pentazocine's effects in the midbrain (Table 2). On the other hand, the depletion of hypothalamic NE after pretreatment with naloxone was actually greater ($P < 0.05$)

TABLE 4. EFFECTS OF PENTAZOCINE AND NALOXONE ON SEROTONIN CONTENT OF DISCRETE REGIONS OF RAT BRAIN*

| Dose (mg/kg) | | Regional 5-HT content (ng/g of tissue) | | | | |
|---|---|---|---|---|---|---|
| Naloxone | Pentazocine | Cortex | Hypothalamus | Midbrain | Medulla | Whole brain |
| 0 | 0 | 376 ± 12 | 1138 ± 74 | 567 ± 40 | 667 ± 32 | 555 ± 24 |
| 0 | 5·6 | 356 ± 47 | 982 ± 66 | 619 ± 79 | 727 ± 35 | 493 ± 30 |
| 0 | 17·5 | 329 ± 29 | 1025 ± 33 | 439 ± 67 | 659 ± 30 | 468 ± 32 |
| 0 | 56 | 315 ± 25 | 831 ± 85 | 337 ± 38‡ | 563 ± 42 | 489 ± 58 |
| 10 | 0 | 351 ± 26 | 971 ± 52 | 538 ± 31 | 704 ± 11 | 514 ± 51 |
| 10 | 5·6 | 380 ± 18 | 1013 ± 185 | 614 ± 27 | 510 ± 37† | 569 ± 17 |
| 10 | 17·5 | 351 ± 25 | 994 ± 134 | 607 ± 36 | 469 ± 29‡ | 553 ± 25 |
| 10 | 56 | 330 ± 14 | 955 ± 167 | 559 ± 33 | 507 ± 30‡ | 550 ± 23 |
| P value, pentazocine (5·6, 17·5, 56 mg/kg) vs naloxone + pentazocine | | NS | NS | 0·01 | 0·001 | NS |

* Rats were killed 65 min after s.c. administration of naloxone and 60 min after pentazocine. The values presented for the whole brain are exclusive of the cerebellum. Each value represents a mean ± 1 S. E. M. of at least six observations. NS = not significant.

† Significantly different from vehicle-treated controls (top line), $P < 0.05$.

‡ Significantly different from vehicle-treated controls (top line), $P < 0.01$.

than was observed after the administration of only pentazocine. Naloxone had no effect on the depletion of NE caused by pentazocine in the medulla. At the level of the whole brain, it was not possible to demonstrate a significant interaction between naloxone and pentazocine (Table 2). Contrariwise, a significant attenuation of the depletion of DA by pentazocine after pretreatment with naloxone was consistently observed in the cortex and striatum as well as in the whole brain (Table 3).

Although pentazocine alone had little effect on brain 5-HT levels, pretreatment with naloxone resulted in a significant lowering in the content of medullary 5-HT by all of the doses of pentazocine tested (Table 4). However, naloxone did antagonize the previously described pentazocine-induced depletion of 5-HT in the midbrain.

The principal findings of this study are summarized in Table 5.

TABLE 5. SUMMARY OF THE EFFECTS OF PENTAZOCINE AND NALOXONE ON MONOAMINE CONTENT OF DISCRETE REGIONS OF RAT BRAIN*

| | NE | | DA | | 5-HT | |
|---|---|---|---|---|---|---|
| Brain region | Pentazocine | Naloxone + pentazocine | Pentazocine | Naloxone + pentazocine | Pentazocine | Naloxone + pentazocine |
| Cortex | Depletes | Blocks | Depletes | Blocks | No effect | |
| Striatum | Not determined | | Depletes | Blocks | Not determined | |
| Hypothalamus | Depletes | Enhances | Not determined | | No effect | |
| Midbrain | Depletes | Blocks | No effect | | Depletes | Blocks |
| Medulla | Depletes | No effect | No effect | | No effect | Depletes |
| Whole brain | Depletes | No effect | Depletes | Blocks | No effect | |

* Rats were killed 65 min after the administration of naloxone (10 mg/kg) and 60 min after pentazocine (5·6, 17·5 and 56 mg/kg). The naloxone + pentazocine column shows if naloxone blocks, enhances or has no effect on the pentazocine-induced depletion of a brain monoamine.

## DISCUSSION

The effects of pentazocine on the NE, DA and 5-HT content of discrete regions of the rat brain are consistent with the previously reported effects of this drug on the monoamine content of the whole brain.[6] NE and DA levels were lowered by pentazocine over a 10-fold range of doses. NE levels were decreased throughout the brain, whereas pentazocine's effects on DA levels were confined to the cortex and striatum, the two regions with the highest content of DA. The brain section referred to as cortex includes neocortex as well as limbic areas, which have an especially high content of DA. From the present data it is not possible to determine if the site of pentazocine's DA-depleting effect is localized in limbic structures or is general throughout all cortical areas. The effect of pentazocine on brain 5-HT concentration was limited to the midbrain and was of a smaller magnitude than its effects on the catecholamines.

In the whole brain, it was possible to demonstrate significant depletion of catecholamines only at dose levels of at least 16 mg/kg of pentazocine.[6] However, analysis of drug effects in discrete regions of the brain reveals that pentazocine is at least three times more potent in lowering brain DA levels than was previously reported. Thus, depletion of brain DA can be demonstrated at a dose of pentazocine that is less than $\frac{1}{20}$ of the drug's subcutaneous $LD_{50}$ in the rat.[16]

The value of assessing drug effects in discrete brain regions as opposed to the whole brain is especially evident in the experiments with naloxone. Five-min pretreatment with naloxone antagonized the pentazocine-induced depletion of NE in the cortex and midbrain. However, a significant antagonism could not be detected at the level of the whole brain, concordant with an earlier report,[6] most probably because naloxone not only fails to antagonize the depletion of NE produced by pentazocine in the medulla but actually enhances the depletion of hypothalamic NE. On the other hand, the capacity of naloxone to reduce significantly the depletion of DA by pentazocine is at variance with the findings of our previous study.[6] This discrepancy is difficult to reconcile, since comparable procedures were employed in both studies.

The enhancement of pentazocine-induced amine depletion (hypothalamic NE and medullary 5-HT) in the presence of naloxone may seem incongruous with the general conception of naloxone as an antagonist of both pure (i.e. morphine) and partial (i.e. pentazocine) narcotic agonists. However, such an effect is not without precedent in the rat. We have observed naloxone to enhance the stimulation of locomotor activity produced by cyclazocine,[17] a potent narcotic antagonist and structural analogue of pentazocine.

If the agonistic actions of narcotic antagonists can be classified as specific or nonspecific based upon whether or not these actions are blocked by a pure antagonist such as naloxone,[18] then it becomes apparent that the effects of pentazocine on the monoamine content of the rat brain involve both a specific and a nonspecific component. A possible weakness in this argument resides in the fact that only a single dose of naloxone was tested at one time point. However, the time of testing was well within the duration of naloxone's antagonistic activity,[8,19] and the dose level of naloxone was one to two orders of magnitude greater than that necessary to antagonize the analgesic effect of pentazocine.[9,19] Finally, these experiments have demonstrated that the specific component of action of pentazocine can remain

undetected when drug effects on monoamine content are evaluated at the level of the whole brain.

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