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BRIEF COMMUNICATION

Propranolol, ^{14}C-Morphine Accumulation and Avoidance: Peripheral and Central Variables[1]

W. C. BLACK, W. J. MCBRIDE AND H. J. GROSZ

Institute of Psychiatric Research, Department of Psychiatry, Indiana University School of Medicine Indianapolis, IN 46202

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BLACK, W. C., W. J. MCBRIDE AND H. J. GROSZ. *Propranolol, ^{14}C-morphine accumulation and avoidance: peripheral and central variables.* PHARMAC. BIOCHEM. BEHAV. **3**(4) 701–704, 1975. – Pretreatment with propranolol HCl resulted in a substantial reduction in the amount of ^{14}C-morphine found in blood plasma, neostriatum plus adjacent tissue, pons-medulla, midbrain, cerebral cortex and cerebellum 25 min after IP injection of the labelled morphine. Previous observations, demonstrating attenuation of behavioral effects of morphine following pretreatment with propranolol, might have been due to a decrease in peripheral and/or central levels of morphine. However, when morphine HCl (10–20 μM) was administered via intraventricular cannulae following pretreatment with propranolol given intraperitoneally in rats performing free operant (Sidman) avoidance, the anti-avoidance effects of the opiate were also attenuated. Hence, previous results cannot be attributed solely to reduced concentration of morphine at CNS site(s) of action.

Avoidance Propranolol Morphine Ventricular administration Drug interactions

CLINICAL and basic studies have demonstrated interaction between propranolol, ((D,L-(isopropylamine)-3-(1-naphthyloxy)-2-propanol)) and opiates. The isoproteronal derivative has been shown to prevent heroin-induced euphoria, cause the use of heroin to precipitate protracted withdrawal symptoms and abolish or greatly attenuate craving for narcotics which human ex-addicts commonly experience in the abstinent state [6–9]. Subsequently it was shown that morphine-uptake is inhibited by propranolol in mouse brain, in vitro [3] and recently, that the effects of morphine on conditioned suppression and free operant (Sidman) avoidance are attenuated by pretreatment with propranolol and/or its stereoisomers [2]. The present studies represent an extension of the previous work and are addressed to the assessment of possible peripheral and central mechanisms of interaction between the two compounds [2].

EXPERIMENT 1

METHOD

Animals

Ten male hooded rats (Long-Evans, Blue Spruce Farms, Inc., Altamont, N. Y.), weighing 325–450 g were assigned to two groups (N = 5) on the basis of approximately matched body weights. They were maintained in the animal quarters for 3 weeks under a 12-hr light/dark cycle with free access to food and water prior to the experiment.

Procedure

As in previous behavioral studies [2], 40 min prior to the administration of morphine experimental animals were administered propranolol hydrochloride (15 mg/kg, IP) and controls received 0.9% normal saline in equivalent volumes. Subsequently, all rats were injected (9 mg/kg, IP) with radioactive morphine solution with specific activity of 1.52 mC/mmol. This solution was prepared by dissolving 250 μC of (N-methyl-^{14}C) morphine hydrochloride (specific activity = 54 mC/mmol; Amersham-Searle, Arlington Heights, Ill.) in 4.0 ml unlabelled morphine sulphate (15 mg/ml). The animals were decapitated 25 min after administration of morphine, blood samples were taken and the brains were removed. The 15 mg/kg dose of propranolol was selected for this particular experiment following several previous attempts to demonstrate the phenomenon in which minimal but consistent differences were observed at specific

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activities between 0.7–0.9 mC/mmol. For the purpose of this demonstration, therefore, the dose of propranolol was doubled and the specific activity of the ^{14}C-morphine was increased to enhance the sensitivity of the assay. Decapitation at 25 min following morphine was carried out because it approximates the time course of maximum analgesia reported in other work [12]. The following brain parts were then dissected on dry ice: corpus striatum, amygdala and septum were pooled and assayed as one sample; the midbrain; slices of cerebral cortex; cerebellum; and pons-medulla oblongata.

Brain parts were then weighed and homogenized in 1M NH_4OH and the unbound radioactive morphine subsequently extracted into 10% (v/v) n-amyl alcohol in ethylene chloride [12]. Radioactivity was measured with a scintillation counter equipped with external standardization to correct for quenching (Packard Tri-Carb Model 3375; Packard Instruments Co., Downers Grove, Ill.).

RESULTS

As represented in Fig. 1, ^{14}C-morphine accumulation in blood plasma and all brain regions sampled was significantly diminished by pretreatment with propranolol ($p<0.05$ as analyzed with Student's t statistic for independent samples). The initial N was reduced by accidental loss of some samples. Of the regions sampled, the 90 percent difference manifested in plasma accumulation was approximated only by that in the cerebellum. The smallest relative difference, obtained in the striatum and adjacent tissue, approximated 70 percent. These results suggested that previous data [2] might have been the result, in part, of peripheral phenomena associated with the entry of morphine into the circulation, either by the alteration of peritoneal membrane permeability by virtue of the "quinidine-like" properties of propranolol [5] or by alteration of binding of the opiate to constituents of the blood [4,17] by propranolol. Competitive interference with binding is unlikely because it would probably be reflected in elevations of unbound morphine as measured in the present study, an effect which is conspicuously absent. However, it might be that unbound, or free morphine, would be more susceptible to accelerated or altered metabolism. Such a potential effect of propranolol has not been investigated as yet. The present study offers no evidence of the distribution or disposition of bound morphine. Considering the numerous possibilities for peripheral mechanisms, the following experiment was carried out.

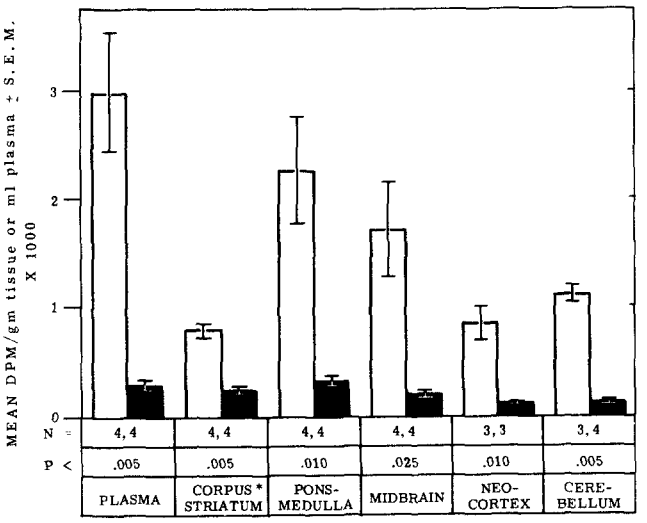

FIG. 1. The effects of pretreatment with propranolol HCl (solid bars) with 15 mg/kg, IP upon accumulation of ^{14}C-morphine. Control animals received 0.9% normal saline, IP on an equal volume/body weight basis (open bars). *Corpus striatum samples included portions of adjacent amygdala and septum. Brackets on each column represent standard error of the mean.

EXPERIMENT 2

METHOD

Animals

Eight male, hooded rats (Long-Evans, Blue Spruce) weighing 400–500 g were maintained with free access to food and water. Training was started after they had been in the local colony for at least 3 weeks under the standard 12 hr light/dark regimen.

Apparatus

The experimental chamber consisted of a Plexiglas and aluminum cage (23.5 cm wide, 20.2 cm deep, 19.2 cm high) fitted with a microswitch lever placed 2.0 cm above the grid floor. The grids were spaced 1.5 cm apart. Shock was delivered to the grids, the metal sides of the cage, and the lever from a commercial, scrambled source (Grayson Stadler No. E1064 or No. 700). A pilot light 7.5 cm above the lever was illuminated during avoidance sessions. Each cage was enclosed in a sound-attenuating chamber.

Procedure

The rats received 15 daily training sessions of 4–5 hr duration in free operant (Sidman) avoidance. The schedule parameters were: shock-shock interval = 5 sec, response-shock interval = 20 sec. Responses and shocks were recorded every 5 min by means of a printout counter. Performance was considered asymptotic when the animals received no more than 8 shocks/5 min period. Shock parameters were maintained throughout training and experimental sessions at 1.0 mA X 0.5 sec. Following the 15th training session, each animal was implanted stereotaxically with a unilateral cannula, aimed for the lateral ventricle, under sodium pentobarbital (55 mg/kg, IP) [14]. Seven to 10 days after surgery, daily 5 hr avoidance sessions were resumed with 0.9% normal saline or propranolol HCl (7 mg/kg) administered IP at 75 min, and intraventricular injections of artificial cerebrospinal fluid (CSF) [17] administered at 150 min and at 225 min. Intraventricular injections were delivered in 5 μl volumes over a period of 30 sec by means of a motor-driven micrometer-pump of local construction, which activated a Hamilton microliter syringe. This postsurgical regimen was continued for 10 daily sessions to accommodate the animals to repeated interruptions of the session and to control for injection effects. Each animal was then tested for morphine sensitivity. Morphine was administered at the 225th min of the session at concentrations of 5, 10 or 20 mM in a 5 μl volume of CSF (with pH adjusted to 7.2 by bubbling with 20% CO_2). The dose appropriate for each animal was determined as the concentration which demonstrably altered

response rate and shock density without abolishing behavior altogether. Ten or 20 mM was established as the test concentration for the animals discussed here. In order to minimize the development of tolerance, morphine was administered at intervals of no less than 7 days.

The interval between the administration of propranolol and morphine ranges from 40 min in Experiment 1 to as much as 150 min in Experiment 2. In the original demonstration of this phenomenon on avoidance [2] 80 min interinjection interval was selected because that much time was deemed necessary to obtain a minimum adequate sample of avoidance behavior after each injection. In all the avoidance experiments the first 80 min are obtained without pharmacological or control manipulation, the data being considered behavioral warm-up time. The optimal premorphine interval for the injection of propranolol has not been established as yet in the rat.

RESULTS

Intraperitoneally administered propranolol attenuated the anti-avoidance effects of intraventricularly administered morphine. The behavior and its consequences, as represented in Fig. 2 are typical of the data for all animals in this study. Neither propranolol alone, nor intraventricular CSF produced significant alteration of behavior. Note that the number of shocks remained fairly constant in the control sessions despite the variability in response rate.

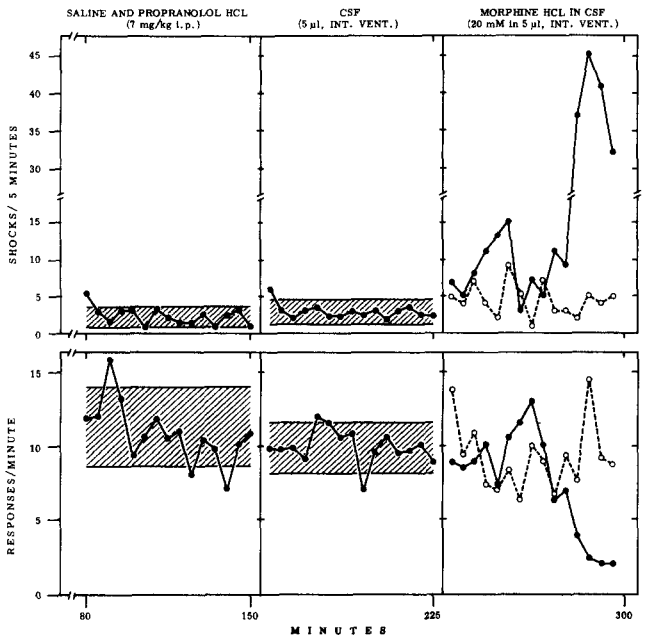

FIG. 2. The effects upon free operant avoidance performance of intraventricular morphine HCl on one animal. The dashed line in the third panels indicates sessions in which the animal received pretreatment with propranolol. The shaded areas in the first 2 panels indicate one standard deviation for the average of 2 sessions, while the third panel represents 2 sessions with the different pretreatments.

An interesting feature of the effects of morphine on avoidance is reflected in the first 45 min following its administration. Response rate changed very little in comparison to control rates during this period, but the ineffectiveness of the behavior was reflected in a substantial increase in the number of shocks the animal received. In the last 30 min of the 75 min period following morphine, the response rate diminished precipitously with a concomitant sharp increase in shock density. When the sequence of propranolol and CSF was interchanged prior to the administration of morphine, changes of the same relative magnitude, both in response rate and shock density, were observed. In Fig. 3, the total session length was prolonged, and the last 40 min of each segment is plotted. In this case, the animal was more sensitive to morphine. The effect of propranolol was to sustain response rate at a level closer to that observed in controls. Note, however, that although the shock density was much lower than that obtained in the absence of propranolol, the sustained response rate did not yield a reduction in shock density comparable to controls.

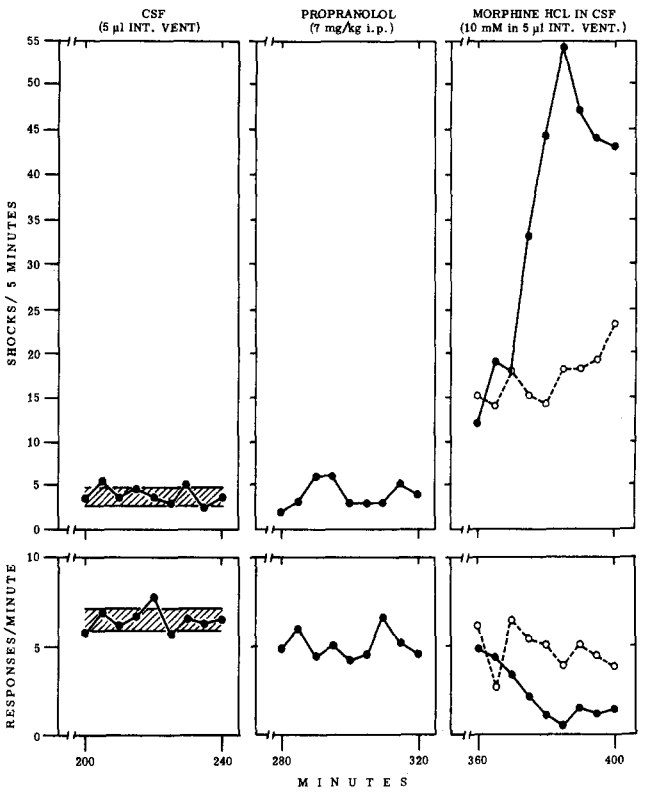

FIG. 3. The effects upon free operant avoidance performance of intraventricular morphine HCl depicting results obtained in the last 40 min of 3 consecutive 80 min segments of a session. In this case the sequence of the intraventricular CSF control and intraperitoneal propranolol was reversed. Propranolol was administered at 240 min; morphine was administered at 320 min.

DISCUSSION

These studies have several implications for further study of the mechanisms of interaction between propranolol and morphine. At present, there is no evidence for the assumption that the compounds interact competitively at central synaptic sites. The data of Experiment 1, demonstrating reduced plasma and tissue levels of unbound morphine following propranolol treatment, suggest a substantial periph-

erally-mediated component which might prevent the delivery of morphine to opiate receptors in the brain. The degree to which propranolol might alter morphine metabolism and excretion remains unknown at present. The differences in plasma accumulations may be related to data demonstrating that both propranolol [4] and methadone are bound to blood proteins [15]. However, if competitive interaction were involved at this level, propranolol might potentiate some of the activities of morphine by increasing the amount of free morphine in the plasma available to reach the CNS. Indeed, potentiation of heroin effects has been observed in clinical use [7,8] and has been demonstrated when propranolol is given at doses larger than 15 mg/kg prior to morphine in acute animal studies (unpublished observations). The significant differences in amounts of morphine in different regions of the brain and the possible relevance of these differences to propranolol/opiate interaction require further investigation.

The data presented in Experiment 2 provide support for the role of central variables in interaction of the two compounds. The time course of behavioral effects following sequential administration (morphine after propranolol) is similar whether morphine is administered intraperitoneally or intraventricularly, and propranolol attenuates the effects of morphine in both paradigms [2].

Autoradiographic studies of both propranolol [13] and morphine [18] which demonstrate accumulation of these compounds in the substantia nigra, caudate, putamen, and morphine accumulation in hypothalamic structures bordering the third ventricle [18] offer circumstantial evidence of potential central sites of interaction. Direct application of morphine to ventromedial sites has been observed to produce analgesia, whereas application to dorsolateral sites within the diencephalon produced hyperalgesia or no effect [11]. Observations that opiate receptors are conspicuous in the neostriatum [16], that this area is characterized by a disproportionate number of dopaminergic nerve endings [1] and that morphine seems to have a preferential effect upon dopaminergic dynamics [10] suggest additional possibilities for central interaction since propranolol reportedly enhances the activity of tyrosine hydroxylase in the striatum [19]. Several additional central, non-synaptic possibilities remain to be investigated; that is, that propranolol may interfere with the passage of morphine from the ventricles to neural tissue, or that the membrane stabilization properties of propranolol [5] are exerted directly upon neuronal membranes in situ, thus reducing the uptake of morphine to sites of action.

REFERENCES

1. Anden, N.-E., A Dahlstrom, K. Fuxe and K. Larsson. Functional role of the nigro-neostriatal dopamine neurons. *Acta Pharmac.* **24:** 263–274, 1966.
2. Black, W. C. and H. J. Grosz. Propranolol antagonism of morphine-influenced behavior. *Brain Res.* **65:** 362–367, 1974.
3. DeFeudis, F. V. and H. J. Grosz. Effects of propranolol on the binding of ^{14}C-morphine to particulate fractions of mouse brain. *Brain Res.* **47:** 510–514, 1972.
4. Evans, G. H., A. S. Nies and D. G. Shand. The disposition of propranolol. III. Decreased half-life and volume of distribution as a result of plasma binding in man, monkey, dog, and rat. *Pharmac. exp. Ther.* **186:** 114–122, 1973.
5. Goodman, L. S. and A. Gilman. *The Pharmacological Basis of Therapeutics.* New York: MacMillan Co., 1970, pp. 566–567.
6. Grosz, H. J. Successful treatment of a heroin addict with propranolol: implications for opiate addiction treatment and research. *J. Indiana Med. Ass.* **65:** 505–509, 1972.
7. Grosz, H. J. Narcotic withdrawal symptoms in heroin users treated with propranolol. *Lancet* **2:** 564–566, 1972.
8. Grosz, H. J. Effect of propranolol on active users of heroin. *Lancet* **2:** 612, 1973.
9. Grosz, H. J. Propranolol in the treatment of heroin dependence. *Drug dependence-treatment and treatment evaluation,* Proc. Skandia Intern. Symp., Stockholm, 1974.
10. Gunne, L.-M., J. Jonsson and K. Fuxe. Effects of morphine intoxication on brain catecholamine neurons. *Eur. J. Pharmac.* **5:** 338–342, 1969.
11. Jacquet, Y. K. and A. Lajtha. Morphine action at central nervous system sites in rat: analgesia or hyperalgesia depending on site and dose. *Science* **182:** 490–492, 1973.
12. Johannesson, T. and L. A. Woods. Analgesic action and brain and plasma levels of morphine and codeine in morphine tolerant, codeine tolerant and non-tolerant rats. *Acta pharmac. tox.* **21:** 381–396, 1964.
13. Masuoka, D. and E. Hansson. Autoradiographic distribution studies of adrenergic blocking agents. II. ^{14}C-propranolol, a beta-receptor-type blocker. *Acta pharmac. tox.* **25:** 447–455, 1967.
14. Myers, R. D., G. Casaday and R. B. Holman. A simplified intracranial cannula for chemical stimulation or long-term infusion of the brain. *Physiol. Behav.* **2:** 87–88, 1967.
15. Olsen, G. D. Methadone binding to human plasma albumin. *Science* **176:** 525–526, 1972.
16. Pert, C. B. and S. H. Snyder. Opiate receptor: demonstration in nervous tissue. *Science* **179:** 1011–1014, 1973.
17. Reed, D. J., G. D. Withrow and D. M. Woodbury. Electrolyte and acid-base parameters in rat cerebrospinal fluid. *Expl. Brain Res.* **3:** 212–219, 1967.
18. Schubert, P., H. Teschemacher, G. M. Kreutzberg and A. Herz. Intracerebral distribution pattern of radioactive morphine and morphine-like drugs after intraventricular and intrathecal injection. *Histochemie* **22:** 277–288, 1970.
19. Sullivan, J. L., D. S. Segal, R. J. Kucenski and A. J. Mandell. Propranolol induced rapid activation of rat striatal tyrosine hydroxylase concomitant with behavioral depression. *Biol. Psychiat.* **4:** 193–203, 1972.