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BRIEF COMMUNICATION

Handling and/or Saline Injections Alter Basal and Morphine-Evoked Changes in Dopamine Metabolites in the Striatum and Nucleus Accumbens of Rats

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JOHNSON, D. W. AND S. D. GLICK. *Handling and/or saline injections alter basal and morphine-evoked changes in dopamine metabolites in the striatum and nucleus accumbens of rats.* PHARMACOL BIOCHEM BEHAV 47(3) 765–768, 1994. – The effect of IP saline injections on basal dopamine (DA), homovanillic acid (HVA), and dihydroxyphenylacetic acid (DOPAC), and morphine-evoked levels of DA, DOPAC, and HVA in the striatum (STR) and nucleus accumbens (NAC) of female Sprague–Dawley rats was quantified, using in vivo microdialysis. In the STR, saline injections twice daily over 3 days, then once on day 4 (2×/day group), resulted in higher basal DOPAC and HVA levels compared to unhandled rats (naive group), or rats injected with saline once daily for 4 days (1×/day group). DOPAC and HVA were greater after IP injection of 30 mg/kg morphine in the saline 2×/day group compared to the saline 1×/day and naive groups. In the NAC, DOPAC was higher after injection of morphine in saline-pretreated rats, compared to naive rats. These results suggest that handling and/or IP saline injections affect basal and morphine-evoked changes in DA metabolites in the STR, and morphine-evoked DOPAC in the NAC.

Handling Morphine Nucleus accumbens Striatum Microdialysis Dopamine HVA DOPAC

IT has been suggested that the effects of exposure to stressful situations can grow with the passage of time, and that a single stressful event can lead to sensitization or tolerance to subsequent stressors that occur later [see (2), for review]. Indeed, even simple handling of postnatal rat pups permanently alters the response of the hypothalamic-pituitary-adrenal (HPA) axis to a variety of stressful stimuli later in life (17).

A large body of evidence suggests that alteration of DA release and/or metabolism in the mesolimbic system may be one mechanism of how stress imposed in some fashion may later alter the effects of other stressful events, including drug administration (5,8,11,14,19). Stress-induced changes in catecholamine release and/or metabolism in the nigrostriatal pathway are not found as frequently as in the mesolimbic system, but cold water baths, tail shock, tail pinch, and restraint have all been shown to alter DA and DA metabolite concentrations in the nigrostriatal pathway as well (1,7,13).

We (12) recently reported the effects of morphine tolerance on basal and acute morphine-evoked levels of DA, HVA, and DOPAC in the STR and NAC of rats. We now report that prior handling and/or saline injections modifies basal levels of DOPAC and HVA in the STR, and alters morphine-evoked increases of DOPAC and HVA in the STR and morphine-evoked DOPAC in the NAC.

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METHOD

Animals and Pretreatment Regimens

Female Sprague-Dawley rats (250–275 g) were obtained from Charles River (Wilmington, MA), and used in all experiments. Animals were housed in metal cages at 21–23°C, and maintained on Purina rodent chow and tap water ad lib. Lights were on between 0800 and 2000 h. Animals were housed in groups of no greater than six initially, and then individually after microdialysis guide cannula implantation.

Microdialysis Experiments

Under pentobarbital anesthesia (50 mg/kg), all rats used in this study were implanted stereotaxically with guide cannulas in the left NAC and right STR. The coordinates for guide cannula placement in the NAC were: rostral +1.6 mm from bregma, lateral +1.5 mm, and ventral −4.6 mm from the skull surface. For STR, coordinates were: rostral +0.5 mm, lateral −2.9 mm, and ventral −3.0 mm (18). The cannulas were fixed firmly to the skull with four screws and dental cement. A fence of perforated aluminum (Small Parts, Inc. #J-PMA-062) was cemented around the perimeter of the implant area to protect the probes and provide a site of attachment later for the swivel tether.

Two days after surgery, rats were assigned to either no pretreatment (naive group), IP saline injections (1 ml/kg) once daily for 4 days (1 × /day group), or IP saline twice daily for 3 days, then once on day four (2 × /day group). At least 5 h after receiving the final saline injection on day 4 (for rats in the saline groups), rats were lightly anesthetized with methohexital (6 mg/kg), and placed in a cylindrical Plexiglas testing chamber (30 cm diameter). A CMA/12, 3 mm microdialysis probe (Bioanalytical Systems, W. Lafayette, Ind; Cat. #8309563) was lowered into each guide cannula. The tips of the probes were 4 mm lower than the tips of the guides. The probe inlets were connected to a liquid swivel that was tethered to the skull pedestal. All probes were calibrated at least 24 h prior to use in artificial cerebrospinal fluid (aCSF) containing 146 mM Na^+, 2.7 mM K^+, 155 mM Cl^-, 1.2 mM Ca^{++},1 mM Mg^{++}, and 1 μM ascorbic acid, degassed with argon, and warmed to 25°C. The aCSF was delivered by a Harvard syringe pump at a flow rate of 1 μl/min for calibrations and experiments. Probes were reused no more than three times, and recoveries for all compounds were in the 20–35% range.

All experiments were carried out between 0900 and 1600 h the day after rats were placed in the Plexiglas testing chamber. During each experiment the animal's movement was inhibited only by its cable connection from the skull pedestal to the swivel. The collection tubes, containing 2 μl of 5 N perchloric acid solution, were placed in a holder on the swivel tether so the samples could be removed without disturbing the animal. Six 20-min fractions for each brain area were collected as baseline samples. At the end of the 6th baseline sample collection, all rats received an IP injection of 30 mg/kg morphine, and collection of 20-min fractions continued for an additional 3 h. Cannula placements were confirmed at the end of each experiment by standard histological procedures.

Catecholamine Assay

Perfusate samples were measured using a Waters HPLC system consisting of a refrigerated 712 WISP autosampler, 510 pump, and a 464 electrochemical detector. The column was a 3 μM reverse phase column (Phase Seperation Spherisorb Column S3 ODS2; 10 × 4.6 mm). The mobile phase, consisting of 6.9 g/l sodium monobasic phosphate, 250 mg/l heptane sulfonic acid, 80 mg/l disodium EDTA, and 50 ml/l methanol, was adjusted to pH 3.6 with HCl, and pumped at a rate of 1.2 ml/min. A flow cell from the Waters 460 electrochemical detector was used in the 464 detector; it was set at a potential of +0.80 V vs. Ag/AgCl. Chromatograms were integrated, compared to standards, and analyzed using Waters Maxima Software. The approximate sensitivity limits of the assay with these detector settings and this chromatographic separation were 2–3 pg for DA, 5 pg for DOPAC, and 10 pg for HVA.

Statistical Analysis

For all neurochemical data, a two-factor repeated measures analysis of variance (ANOVA), with time as the repeated measure (within factor), and pretreatment as the other (between) factor, was conducted to evaluate pretreatment and time effects across each brain region for each amine. When a significant pretreatment × time effect was detected for a brain region, a one-factor (pretreatment) ANOVA was subsequently conducted using the overall mean from each pretreatment group, followed by a Student-Newman-Keuls test to determine which means were different from each other.

RESULTS

Basal Release of HVA and DOPAC

Table 1 shows that rats most frequently injected with saline (2 × /day group) over 4 days had elevated basal levels of both HVA and DOPAC in the STR ($p < 0.05$). In the NAC, there were no differences in basal DOPAC (2.6 ± 0.3, 4.0 ± 0.9, 2.5 ± 0.4 μM) and HVA (1.7 ± 0.4, 1.8 ± 0.3, 2.3 ± 0.6 μM) between the 1 × /day, 2 × /day, or naive groups, respectively. No consistent changes were found in basal DA levels between groups in either brain region (data not shown).

Morphine-Evoked Release of HVA and DOPAC

Injection of 30 mg/kg morphine resulted in increased DOPAC and HVA ($p < 0.05$ to 0.001), but not DA, in all groups over time in both the NAC and STR. However, the

TABLE 1

BASAL CONCENTRATIONS OF DOPAC AND HVA IN THE STRIATUM OF NAIVE RATS AND RATS PRETREATED WITH SALINE ONCE OR TWICE DAILY

Compound	*n*	Pretreatment	Mean ± SE
			μM
DOPAC	12	Saline (1 × /day)	3.6 ± 0.5
	13	Saline (2 × /day)	5.9 ± 1.1*
	6	Naive	2.9 ± 0.4
			μM
HVA	12	Saline (1 × /day)	3.3 ± 0.4
	13	Saline (2 × /day)	5.0 ± 0.8*
	6	Naive	2.6 ± 0.2

Saline 1 × /day = Saline IP, 1 ml/kg, once daily for 4 days. Saline 2 × /d = Saline IP, 1 ml/kg, twice daily for 3 days, then once on day 4. Naive rats did not receive injections prior to entering dialysis chamber. All rats were dialyzed on day 5. Basal means for both compounds derived from 3–6 baseline samples from each rat.

*Different from both naive and saline 1 × /day ($p < 0.05$).

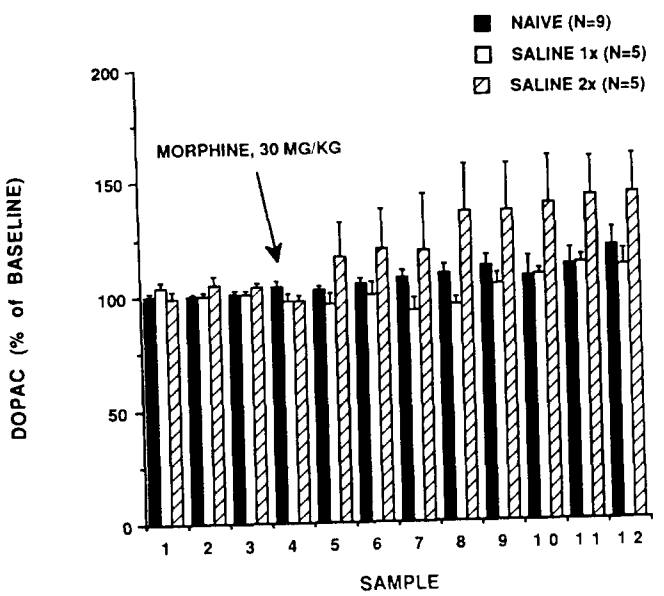

FIG. 1. The effect of IP injection of 30 mg/kg morphine on DOPAC levels in the STR of naive rats, and rats pretreated with saline injections (see the Method section). Morphine was injected immediately after sample 4 was collected. Rats pretreated with saline 2×/day had higher ($p < 0.01$) morphine-evoked DOPAC, compared to both naive rats, and rats receiving saline injections only 1×/day.

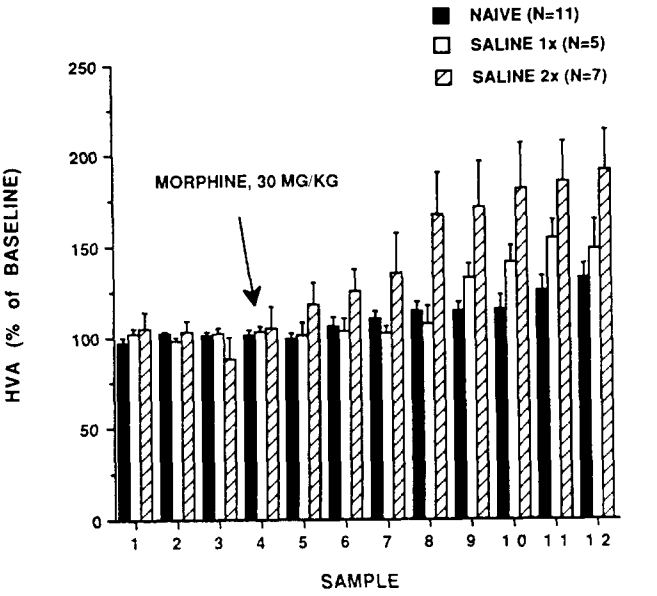

FIG. 2. The effect of IP injection of 30 mg/kg morphine on HVA levels in the STR of naive rats, and rats pretreated with saline injections (see the Method section). Morphine was injected immediately after sample 4 was collected. Rats pretreated with saline 2×/day had higher ($p < 0.05$) morphine-evoked HVA, compared to both naive rats, and rats receiving saline injections only 1×/day.

morphine-evoked increase in DOPAC (Fig. 1; $p < 0.01$) and HVA (Fig. 2; $p < 0.05$) in the STR was higher in rats pretreated with saline 2×/day, compared to naive rats and rats injected with saline 1×/day. In the NAC, morphine increased DOPAC similarly in both groups previously receiving saline injections, but this increase was greater than that observed in naive rats (Fig. 3; $p < 0.05$). HVA, as well as DA, was nearly identical in all groups in the NAC after 30 mg/kg morphine injection (data not shown).

DISCUSSION

Although stress is well known to elevate DA levels in the mesocortical DA-ergic system [see (21), for review], the response of the mesolimbic and nigrostriatal DA-ergic systems to stress is much more variable (1,6,11,13). Despite this variability, in vivo microdialysis and voltammetry studies have clearly demonstrated increases in extracellular DA release from the mesolimbic system after stress (1,7,11), and, to a lesser extent, from the nigrostriatal DA system as well (1, 7,13). Furthermore, it has been demonstrated that previous exposure to stress can modify the extracellular DA response to subsequent stressors in the NAC (5,7,9,10,19), as well as in the STR (7).

The findings reported here suggest that handling and/or IP saline injections 2×/day increases basal DA metabolism in STR, but not in the NAC. A possible explanation for this discrepancy is the finding that in the NAC, increases in extracellular DA provoked by stress are gradually reduced by repeated stress, and essentially dissappear by the fourth day of stress onwards (9). In fact, a study similar to ours detected no differences on either day 1 or day 6 in basal output of DA, DOPAC, and HVA in the NAC of control rats and rats stressed during the 5 days in between samplings (10).

This laboratory (16) has previously shown that a 30 mg/kg

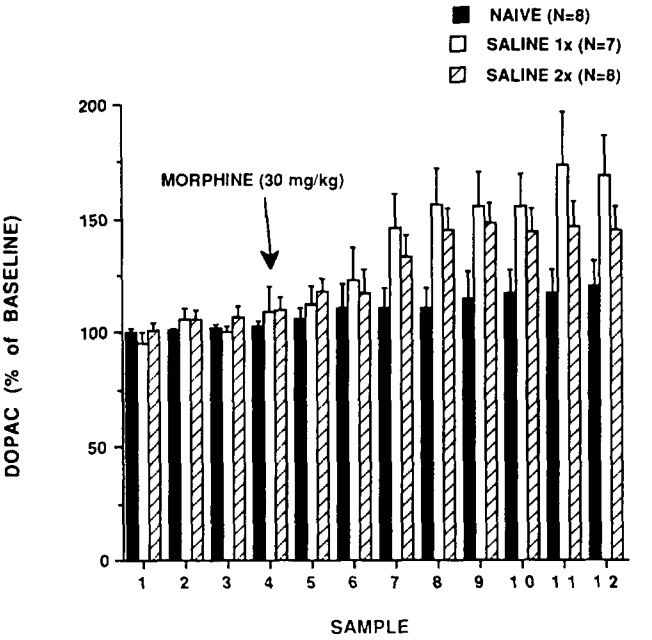

FIG. 3. The effect of IP injection of 30 mg/kg morphine on DOPAC levels in the NAC of naive rats, and rats pretreated with saline injections (see the Method section). Morphine was injected immediately after sample 4 was collected. Rats pretreated with saline 2×/day or 1×/day had higher ($p < 0.05$) morphine-evoked DOPAC, compared to naive rats.

dose of morphine significantly increases levels of DOPAC and HVA, but not DA, over time in the STR and NAC of naive rats, a finding seen in this study as well. Interestingly, however, morphine-evoked increases in DOPAC and HVA were different between groups in the present study. The findings reported here suggest that stress in the form of handling and/or saline injections 2 × /day increases morphine-evoked DOPAC and HVA in STR of rats, and morphine-evoked DOPAC in the NAC of rats handled either 1 × /day or 2 × /day.

One possible explanation for this apparent increase in DA metabolism in the STR and NAC of handled/saline-injected rats is the possibility that these animals have become sensitized to morphine due to the prior stress associated with handling and/or saline injections. In fact, behavioral studies have demonstrated both apparent sensitization and tolerance to drugs when animals have previously been exposed to stressors which themselves were not drugs. For example, a single IP needle jab has been found to alter the cataleptic effects of haloperidol injections in rats 2 weeks later (3), and prior exposure to foot shock has been shown to enhance the locomotor response to subsequent morphine injections in rats (15). Exposure to foot shock also enhanced rotational behavior induced by a later dose of amphetamine (20), and a single saline injection completely abolished the anxiolytic effects of diazepam in rats 1 month later (4). In addition, prior exposure to saline injections has been reported to enhance amphetamine-stimulated DA efflux from striatal slices (22).

In conclusion, this study demonstrates that handling and/or saline injections received by female rats over a 4-day period can alter basal DOPAC and HVA in the STR, and subsequent morphine-evoked increases in DOPAC and HVA in the STR, and DOPAC in the NAC. These results, therefore, suggest that some of the variation reported by different laboratories regarding the postmorphine DA neurochemistry of the STR or NAC, as measured by in vivo microdialysis, may be at least partly due to the amount of routine handling and/or number of saline injections rats were exposed to prior to neurochemical sampling.

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