Role of Central Opioids in Benzodiazepine Modulation of Gustatory Behavior

Alice Tran[1], David Pittman[2], & J.P. Baird[1]

[1]Dept. Psychology, Amherst College, Amherst, MA 01002. [2]Dept. Psychology, Wofford College, Spartanburg, SC, 29301.

INTRODUCTION

- Benzodiazepine receptor agonists (BZR) increase ingestion (Sanger & Blackman, 1976; Cooper & Francis, 1979), though the underlying mechanism is not fully understood. Sham-feeding (Cooper & Kirkham, 1987), taste preference (Cooper & Green, 1993), taste reactivity (Gray & Cooper, 1995), and licking microstructure analysis (Higgs & Cooper, 1998) studies suggest that BZRs enhance taste evaluation.

- Recently, systemic naltrexone (NTX) suppressed a BZR-induced increase in the taste reactivity responses to a quinine-sucrose solution (Richardson et al., 2005). Sub-threshold doses of NTX were without effect, suggesting that opiate and BZ systems interact directly. The site(s) of this interaction remain to be determined.

- We evaluated whether pre-treatment with subthreshold NTX via injections to the forebrain lateral ventricle (LV) or hindbrain fourth ventricle (4V) abolished hyperphagia induced by systemic treatment with the BZR, chlordiazepoxide (CDP). We compared responses after LV and 4V infusion in an effort to being to isolate a region for proposed opiate-BZ system interaction(s). Lick microsructure analysis enables the measurement of perceived taste evaluation and postingestive feedback inhibition through measures of the temporal distribution of licks (Spector, Klumpp & Kaplan, 1998).

METHODS

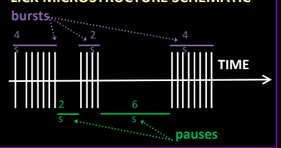
- Male Sprague-Dawley rats were fitted with guide cannulas aimed at the LV or 4V. Cannula placements were confirmed with ink injection and 50ug/5ul angiotensin II (LV) or 90ug/2ul 5TG (4V). Rats were trained to ingest 0.3M sucrose in a lickometer (DiLog Instruments) prior to testing.

- For dose response curves (DRC) , rats fitted with LV (n=4) or 4V (n=7) cannulas had 30-minute, daily access to 0.3M sucrose in lickometer cages. On testing days, rats received a microinjection 15 minutes prior to sucrose access. LV rats received 5 µl injections every third day of one of a range of doses of NTX (5.0, 7.5, 10, 15, 25, 50 µg) or vehicle (artificial CSF). 4V rats received 2 ul injections every 2nd day, of NTX (5, 10, 25, 50, 100 µg) or aCSF. Every rat received every NTX dose once, in counterbalanced sequence.

- For the main experiments, every 3rd day (testing day), rats each received an ip injection of CDP (10 mg/ml, 1 ml/kg) or saline vehicle (1ml/kg), followed by an lCV microinjection (LV (n=10) or 4V (n=7), 10 µg, 1 µg/minute) NTX or aCSF vehicle, prior to intake testing. During 90-minute intake testing, rats were free to drink the tastant offered in the testing cage (4mM saccharin or 0.1M sucrose). Each testing day was intervened by 2 non injection rest days on which rats were free to drink 0.3M sucrose in testing apparatus for 30 minutes.

- *Meal onset:* first lick of a burst containing at least 15 licks
- *Meal size* (ml): product of meal licks (onset to a pause ≥ 600 s by session's average lick volume.
- *Licking burst:* 2+ consecutive licks with no interlick interval > 1 s.
- *Burst size*: number of licks in all bursts in the meal divided by the number of bursts in the meal.

LICK MICROSTRUCTURE SCHEMATIC



RESULTS

NALTREXONE SUPPRESSED INTAKE…

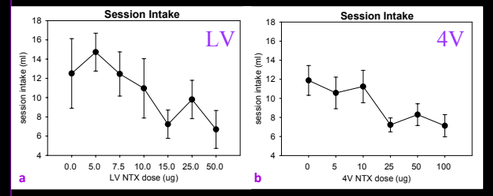


Figure 1. NTX into the LV and 4V dose-dependently suppressed intake. In these DRCs, 10 µg NTX was identified as an appropriate subthreshold dose of NTX.

…BUT NOT THROUGH REDUCED TASTE

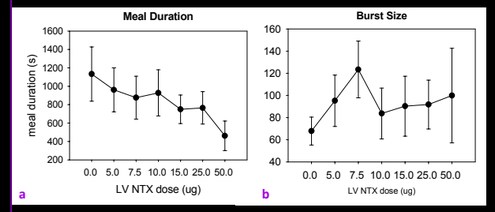


Figure 2. NTX delivered to the LV reduced (a) meal duration and (d) burst number, increased (d) latency, but did not (c) change burst size.

CHLORDIAZEPOXIDE AND LV / 4V NALTREXONE EFFECTS ON LICKING MICROSTRUCTURE

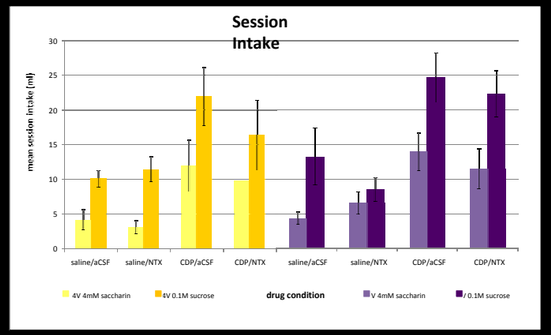


Figure 3. 10 µg icv NTX only modestly attenuated CDP-induced hyperphagia. As expected, CDP increased consumption (p<0.05), while NTX alone did not produce significant differences from vehicle controls. NTX did not significantly offset CDP increases in, except for meal size in the saccharin LV group (p<0.033).

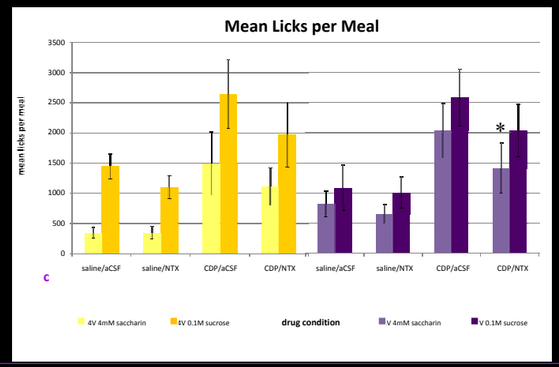
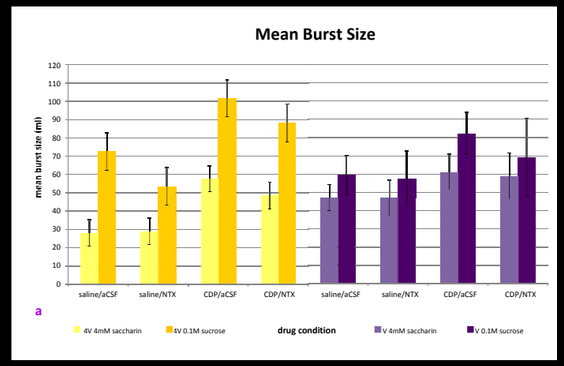


Figure 4. CDP significantly increased the mean burst size and number of bursts in the meal. NTX had no significant effect on these measures when applied alone. NTX only offset CDP-induced increase in burst count for 0.1M sucrose after 4V injection (p<0.05). NTX did not attenuate CDP effects in any other conditions.



DISCUSSION

• In the LV NTX dose response curve, high doses of NTX suppressed sucrose intake by reducing burst number rather than burst size. The lack of effect on burst size suggests that NTX did influence hedonic taste evaluation. The results also suggest that NTX may act through hindbrain sensitive sites.

• A reduction in the number of licking bursts also emerged from the CDP/NTX, 4V sucrose data. The stronger burst number reduction in the 4V injected group calls attention to a possible interaction of CDP and NTX in hindbrain sites. The divergence of sucrose and saccharin responses here suggests that post-ingestive effects such as caloric value may play a role in opioid-antagonist intake modulation. These findings are not unprecedented (Pasquale & Sclafani, 2002) but were surprising in light of previous studies (Rockwood & Reid, 1982; Parker, Maier, Rennie, Crebolder, 1992).

• NTX, a mixed mu/delta antagonist (Cooper, Bloom, Roth, 2002), may not be the optimal opioid antagonist to study opioid-benzodiazepine interactions in feeding behavior. It may be fruitful to focus on the kappa opioid receptor in particular, given evidence reported for kappa rather than mu opioid receptor influences on sucrose sham-feeding (Leventhal & Bodnar, 1996).

• These results suggest that hindbrain sites are pertinent to the opioid – BZR interactions, and that opioid antagonists may be mediating BZR-induced hyperphagia through means other than modulation of orosensory evaluation alone. We propose to further explore the potential roles of forebrain versus hindbrain opioid sites in BZR palatability effects by revisiting these experiments with larger sample sizes. Other questions include whether use of subthreshold kappa opioid antagonist might be more selective in mediating BZR-induced hyperphagia.

REFERENCES

Cooper, J.R., Bloom, F.E., Roth, R.H. (2002). The biochemical basis of neuropharmacology (8th ed.). Oxford UP.

Cooper, S.J., & Francis, R. L. (1979). Effects of acute or chronic administration of chlordiazepoxide on feeding parameters using two food textures in the rat. Journal of Pharmacy and Pharmacology, Nov;31(11):743-6.

Cooper, S.J., & Green, A.E. (1993). The benzodiazepine receptor partial agonists, bretazenil (Ro 16-6028) and Ro 17-1812, affect saccharin preference and quinine aversion in the rat. Behavioural Pharmacology, Feb;4(1):81-85.

Cooper, S.J., & Kirkham, T.C. (1987). Adrenalectomy and the anorectic effects of benzodiazepine inverse agonists and opiate antagonists in rats fed a palatable diet. 40(4):479-82.

Higgs, S., & Cooper, S.J. (1998). Effects of benzodiazepine receptor ligands on the ingestion of sucrose, intralipid, and maltodextrin: an investigation using a microstructural analysis of licking behavior in a brief contact test. Behavioral Neuroscience. Apr;112(2):447-57.

Leventhal, L., & Bodnar, R. J. (1996). Different central opioid receptor subtype antagonists modify maltose dextrin and deprivation-induced water intake in sham feeding and sham drinking rats. Brain Research, Nov 25;741(1-2):300-8.

Parker, L.A., Maier, S., Rennie, M., Crebolder, J. (1992). Morphine- and naltrexone-induced modification of palatability: analysis by the taste reactivity test. Behavioral Neuroscience, Dec;106(6):999-1010.

Pasquale, G.F., & Sclafani, A. (2002). Naltrexone suppresses the late but not early licking response to a palatable sweet solution: opioid hedonic hypothesis reconsidered. Pharmacology Biochemistry and Behavior, Dec;74(1), 163-172.

Richardson, D.K., Reynolds, S.M., Cooper, S.J., Berridge, K.C. (2005). Endogenous opioids are necessary for benzodiazepine palatability enhancement: naltrexone blocks diazepam-induced increase of sucrose-'liking'. Pharmacology Biochemistry and Behavior. Jul;81(3):657-63.

Rockwood, G.A., & Reid, L.D. (1982). Naloxone modifies sugar–water intake in rats drinking with open gastric fistulas. Physiology and Behavior, 29, 1175–1178.

Sanger, D.J., & Blackman, D. E. (1976). Effects of diazepam and ripazepam on two measures of adjunctive drinking in rats. Pharmacology Biochemistry & Behavior, Aug;5(2):139-42.

Spector, A.C., Klumpp P.A., Kaplan J.M. (1998). Analytical issues in the evaluation of food deprivation and sucrose concentration effects on the microstructure of licking behavior in the rat. Behavioral Neuroscience, Jun;112(3):678-94.

SUPPORTED BY HHMI AND DC 07389