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Nucleus Accumbens Opiate-Dopamine Interactions and Locomotor Activation in the Rat: Evidence for a Pre-Synaptic Locus

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SWERDLOW, N. R., M. AMALRIC AND G. F. KOOB. *Nucleus accumbens opiate-dopamine interactions and locomotor activation in the rat: Evidence for a pre-synaptic locus.* PHARMACOL BIOCHEM BEHAV **26**(4) 765–769, 1987.—Locomotor activation produced by the indirect dopamine (DA) agonist amphetamine is reversed by the opiate-receptor antagonist naloxone. Since amphetamine-stimulated locomotion results from the release of DA within the nucleus accumbens (N.Acc.), it is possible that these effects of naloxone result either from a decrease in the pre-synaptic release of DA within the N.Acc. or from a disruption of the effects of DA at, or distal to, the post-synaptic DA receptor. In the present study, we investigated the effects of naloxone on the locomotor-activating properties of dopamine injected directly into the nucleus accumbens. Naloxone (0–2 mg/kg) had no significant effect of DA-stimulated locomotion; the lowest dose of naloxone tested (0.5 mg/kg) was shown to significantly disrupt the locomotor activation produced by amphetamine (0.5 mg/kg). In separate animals, very high doses of naloxone (5.0 mg/kg) had no significant effect on locomotor activation produced by the DA receptor agonist apomorphine in rats following 6-hydroxydopamine (6OHDA) denervation of the N.Acc. These results indicate that naloxone must disrupt amphetamine-stimulated locomotion through its action pre-synaptic to N.Acc. DA receptors.

Locomotor activity Dopamine Opiates Naloxone Amphetamine Apomorphine
Nucleus accumbens Behavior

SEVERAL studies have demonstrated that blockade of central opiate receptors disrupts the locomotor-activating properties of the indirect DA agonist amphetamine [16,17]. Since amphetamine-stimulated locomotion results from the release of DA from pre-synaptic terminals within the N.Acc. [9], there are several potential sites for opiate-DA interactions responsible for this effect of naloxone. First, it is possible that naloxone-blockade of opiate receptors post-synaptic to N.Acc. DA terminals disrupts amphetamine-stimulated locomotion. Such a blockade might occur at opiate receptors within the N.Acc. [2, 14, 22], or within N.Acc. efferent circuitry critical to the behavioral expression of amphetamine-stimulated locomotion [3, 18, 22].

Alternatively, the ability of nalxone to disrupt amphetamine-stimulated locomotion might result from naloxone-blockade of opiate receptors pre-synaptic to N.Acc. DA terminals. For example, it has been postulated that opiate receptors located on mesolimbic DA terminals exert a tonic facilitatory influence on DA release within the N.Acc. [12]; blockade of these receptors might disrupt amphetamine-stimulated locomotion by decreasing the amount of pre-synaptic DA released by amphetamine within the N.Acc.

If naloxone disrupts amphetamine-stimulated locomotion through its action post-synaptic to N.Acc. DA terminals, then we would predict that naloxone should have similar effects on locomotor-activation produced by infusion of DA directly into the N.Acc. Given this mechanism of post-synaptic opiate-dopamine interactions, we would further predict that naloxone should disrupt the locomotor-activating properties of apomorphine following 6OHDA-denervation of the N.Acc., since this "supersensitive" locomotor activity results from apomorphine-stimulation of post-synaptic DA receptors within the N.Acc. [9,21]. In the present experiments, we tested the effects of naloxone on locomotor activation in rats following peripheral injection of amphetamine, direct injection of DA into the N.Acc., or peripheral injection of apomorphine following 6OHDA denervation of the N.Acc. Naloxone significantly disrupted the locomotor-activating properties of amphetamine, but not of DA or apomorphine. These results support the hypothesis that opiate-DA interactions responsible for naloxone-blockade of amphetamine-stimulated locomotion occur at a locus presynaptic to DA receptors within the N.Acc.

METHOD

Thirty-two male albino Wistar rats (200–250 g, Charles

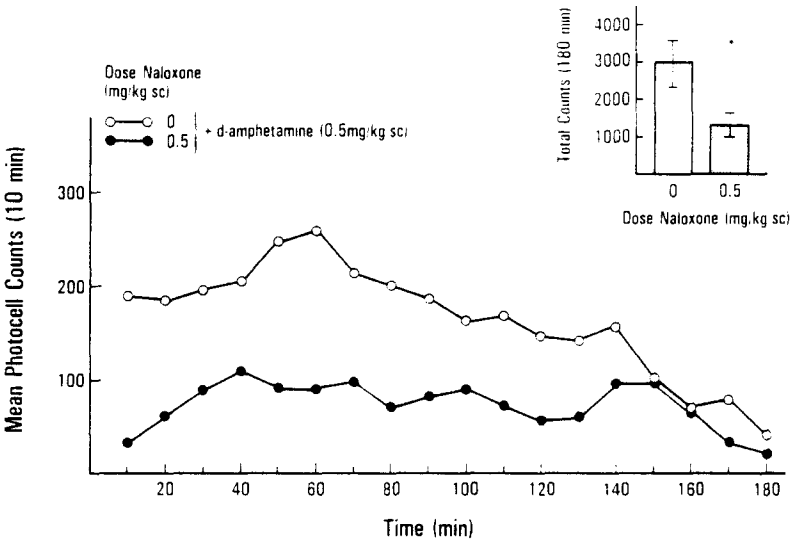

FIG. 1. Locomotor activity in animals following injection of amphetamine (0.5 mg/kg SC). Animals had been pre-injected with either saline (open circles) or 0.5 mg/kg NAL (solid circles). *$p<0.05$, two-way ANOVA with repeated measures on time.

River Labs.) were housed in groups of three, exposed to a normal 12-hr light-dark cycle with free access to food and water, and handled for three min each within three days of shipment arrival.

One week after shipment arrival, twenty animals were anesthetized with pentobarbital (50 mg/kg, IP) and placed in a Kopf stereotaxic instrument with toothbar 5 mm above the interaural line. One group of rats (n=14) received bilateral infusion of 6OHDA (8 μg/2 μl, expressed as free base in 0.1 mg/ml ascorbic acid in saline) through 30 ga cannulae at a rate of 1 μl/3 min aimed at coordinates (from bregma) AP+3.2, L±1.7, DV−7.8 (from skull). A second group of rats (n=6) was implanted with bilateral 23 ga 10 mm steel cannulae aimed 3 mm above the N.Acc. at coordinates (from bregma) AP+3.2, L±1.7, DV−4.8 (from skull), which were fastened with dental cement and filled with wire stylets.

One week after surgery, all operated animals and a third group of unoperated animals (n=12) were familiarized individually to previously-described [6] photocell cages for 180 min. Each cage measured 36×25×20 cm with twin photocell beams across the long axis 2 cm above the cage floor.

One day later, all unoperated animals were habituated to the photocell cages for 90 min. Unoperated animals were injected with naloxone (0.5 mg/kg SC in saline vehicle at a volume of 1 ml/kg; n=6) or saline vehicle (n=6); all animals were then injected with d-amphetamine sulfate (0.5 mg/kg SC in saline vehicle at a volume of 1 ml/kg; doses calculated as salt) and returned to the photocell cages, where their activity was measured for 180 min. This dose of amphetamine was chosen since it has been shown to produce a robust increase in rat photocell activity [6]; the dose of naloxone tested has previously been shown to significantly disrupt amphetamine-stimulated locomotor activity [17].

Animals that received N.Acc. 6OHDA injections were tested on two days, separated by three non-test days. On each test day, animals were habituated to the photocell cages for 90 min. On the first test day, one half of the animals were treated with naloxone (5.0 mg/kg SC) or saline vehicle; all animals were then immediately injected with apomorphine (0.1 mg/kg in saline with 0.1 mg/ml ascorbic acid; subcutaneous injection volume 1 ml/kg) and returned to the photocell cages where their locomotor activity was measured for 90 min. On the second test day, this procedure was repeated, except animals that had received saline now received naloxone, and vice versa. This dose of apomorphine has been shown to significantly potentiate locomotor activity in N.Acc. 6OHDA-injected rats [20].

Animals that were implanted with bilateral cannulae aimed above the N.Acc. were tested on four separate days, with test days separated by three non-test days. On each test day, animals were habituated to the photocell cages for 90 min, and then injected with one of four doses of naloxone (0, 0.5, 1.0 or 2.0 mg/kg SC). All animals received each dose of naloxone only once, and the order of doses over test days was randomized in each animal to control for potential order effects of repeated injections. Following treatment with naloxone, all animals were injected with 40 μg DA (20 μg per side in 1 μl saline vehicle with 0.1 mg/ml ascorbic acid) at a rate of 1 μ/2 min, and returned to the photocell cages where their activity was measured for 180 min. This dose of DA was chosen since it has been shown to produce a reliable increase in rat locomotor activity [11].

Following completion of behavioral testing, animals implanted with intracerebral cannulae were sacrificed by overdose of pentobarbital, and perfused through the heart with cold 10% formalin/saline. The brains were removed and 30 μ frozen sections were cut in a frontal plane using a rotary microtome and stained with cresyl violet. Cannulae sites were assessed without knowledge of the behavioral results.

RESULTS

The locomotor activating properties of amphetamine in saline- and naloxone-pretreated animals are seen in Fig. 1. Two-way analysis of variance (ANOVA) with repeated measures on time revealed that naloxone-injected animals

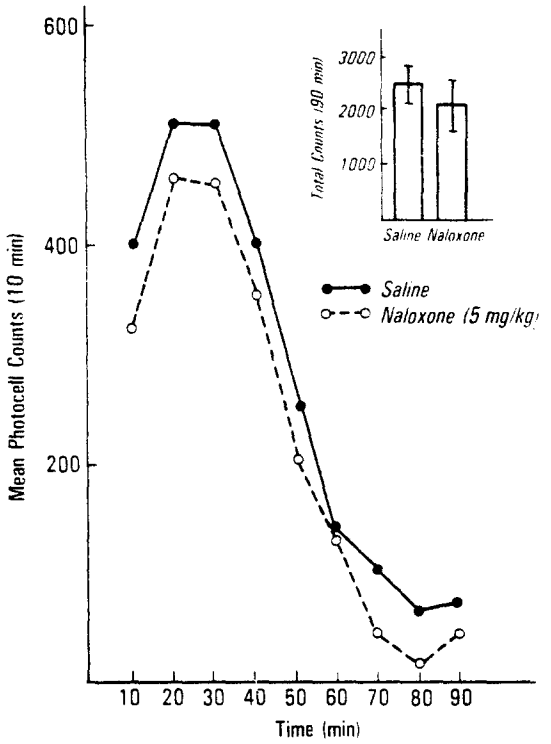

FIG. 2. Locomotor activity in N. Acc. 6OHDA-lesioned animals following injection with apomorphine (0.1 mg/kg SC). Animals had been pre-injected with either saline (solid circles) or 5 mg/kg NAL (open circles).

showed a significantly decreased locomotor response to amphetamine, $F(1,11)=6.17$, $p<0.05$, with no significant treatment (naloxone) × time interaction, $F(17,204)=1.35$, NS. In contrast, locomotor activation following treatment with apomorphine in N.Acc. 6OHDA-injected animals was not significantly decreased by a dose of naloxone ten times greater than that which blocked amphetamine-stimulated locomotion, $F(1,13)<1$, NS, with no significant treatment × time interaction, $F(8,224)<1$, NS (Fig. 2). Infusion of 40 μg DA into the N.Acc. produced a robust increase in locomotor activity equivalent to that produced by 0.5 mg/kg of amphetamine (above). The effects of naloxone on intra-N.Acc. DA-stimulated locomotion were analyzed using a two-way ANOVA with repeated measures on dose and time. Naloxone did not significantly decrease locomotor activation produced by injection of DA into the N.Acc., $F(3,15)=2.04$, NS, with no dose × time interaction, $F(51,255)<1$, NS (Fig. 3). These effects of repeated DA injections demonstrated substantial variability within and between subjects (Fig. 3), but in contrast to the inhibitory effect of naloxone on amphetamine locomotion, the only (non-significant) trend evident from these results is actually a naloxone-enhancement of DA-stimulated locomotion.

Histological analysis of cannula placement revealed a relatively localized distribution of injection sites within the N.Acc. (Fig. 4). No injection sites were localized outside of the N.Acc., nor was significant damage noted to surrounding structures.

DISCUSSION

Numerous studies have confirmed that the locomotor-activating properties of amphetamine in the rat result from the release of DA from pre-synaptic terminals within the N.Acc. and the subsequent activation of post-synaptic DA receptors. Thus, amphetamine-stimulated locomotor activity is disrupted by destruction of mesolimbic DA-containing cell bodies within the ventral tegmental nucleus (VTA) with 6OHDA [10], by destruction of pre-synaptic DA terminals within the N.Acc. with 6OHDA [6,9], by blockade of post-synaptic DA receptors within the N.Acc. with neuroleptic agents [13,14], or by destruction of cell bodies within the N.Acc. that are believed to support the post-synaptic DA receptors [8].

While these dopaminergic substrates of amphetamine-stimulated locomotion have been thoroughly described, it is less clear where opiate-DA interactions might occur to account for the ability of naloxone to disrupt the locomotor-activating properties of amphetamine. As in the striatum [15] opiate receptors in N.Acc. may be located on intrinsic neurons since a proportion of them remain after destruction of the dopaminergic innervation [12], and it is believed that opiates exert their behaviorally-activating and positive-reinforcing properties through their action on these intrinsic N.Acc. opiate receptors [19]. Other opiate receptors have been localized within the ventral globus pallidus, in a region that receives N.Acc. efferent enkephalinergic fibers [3,22]. Infusion of enkephalin compounds into this pallidal region stimulates locomotor activation in rats [7]. It is thus conceivable that naloxone might disrupt amphetamine-stimulated locomotion through blockade of these or other opiate receptors distal to the site of DA release within the N.Acc.

Our present results, however, indicate instead that the critical opiate-DA interactions responsible for the ability of naloxone to disrupt amphetamine-stimulated locomotion occur pre-synaptic to the site of amphetamine-stimulated DA release in the N.Acc. Thus, while we confirmed previous reports that naloxone blocks amphetamine-stimulated locomotor activity [16,17], locomotion stimulated by direct activation of N.Acc. DA receptors with DA or apomorphine is not disrupted by naloxone. Likely sites for this naloxone action thus include opiate receptors localized on dopaminergic N.Acc. afferent fibers or those localized on DA-contained cell bodies within the VTA [12]. Since amphetamine-stimulated DA release in acute preparations is independent of neuronal impulse activity [5], it is unlikely that the observed effects of naloxone result from its action on DA cell bodies in the VTA. A more plausible hypothesis is that naloxone disrupts amphetamine-stimulated locomotion through its action on opiate receptors located on or near pre-synaptic DA terminals within the N.Acc, and that blockade of these receptors decreases the amount of DA released by amphetamine. Such a hypothesis might best be studied using direct measurement of DA release within the N.Acc.

In earlier reports from our laboratory [1,17], we suggested that opiate receptors located on cells within the N.Acc. might be a critical substrate for the locomotor-activating properties of opiate agonists. Thus, heroin-stimulated locomotion is reversed by direct injection of low doses of methyl-naloxonium HCl into the N.Acc. [1], but it is not antagonized by destruction of N.Acc. afferent DA fibers [19]. Heroin-locomotion is not reversed by sub-cataleptic doses of DA receptor-antagonists [19], further suggesting a dissociation between N.Acc. afferent DA systems and the substrates for opiate-activation. Together with our current results, these observations suggest that pre- and post-

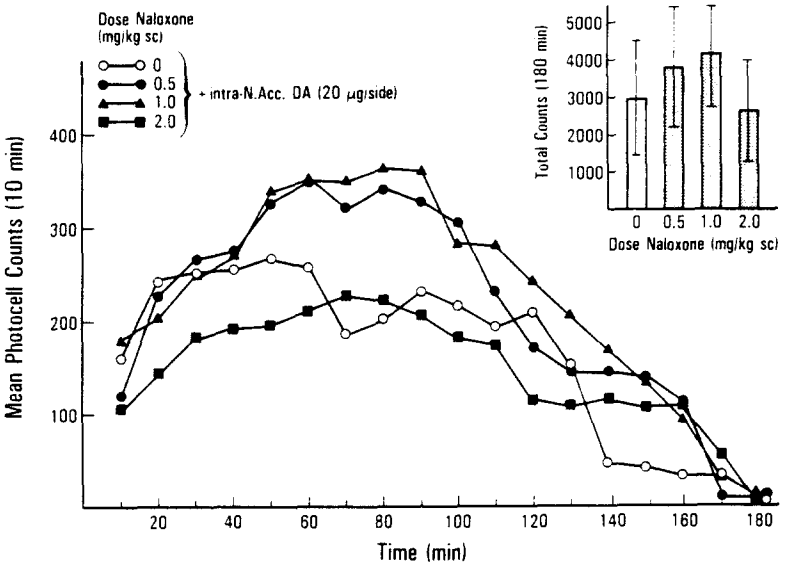

FIG. 3. Locomotor activity in animals following injection of DA (20 µg/side) into the N.Acc. Animals had been pre-injected with one of four doses (0, 0.5, 1.0, 2.0 mg/kg SC) of NAL.

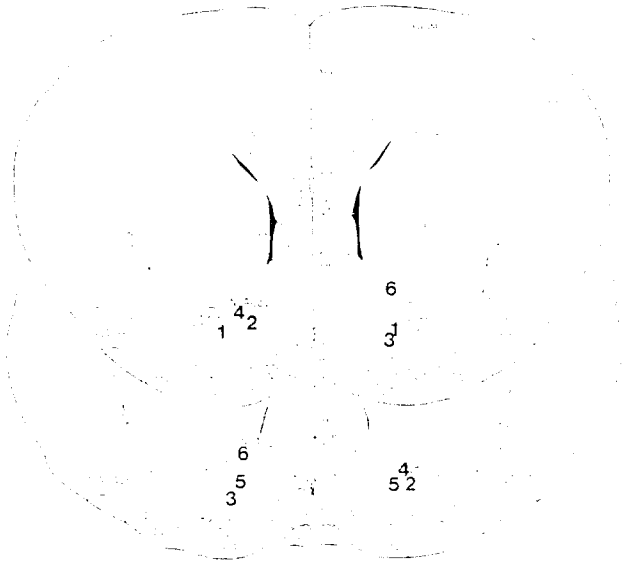

FIG. 4. Distribution of cannulae injection sites within the N.Acc. CA—anterior commissure; CC—corpus callosum; CPU—caudate putamen. Numbers identify particular test animal. Figure adapted from Paxinos, G. and C. Watson, *The Rat Brain in Stereotaxic Coordinates*, Academic Press Inc. (New York, NY) 1982 (Fig. 11–12).

synaptic mesolimbic opiate receptors serve distinct functions. Pre-synaptic opiate receptors located on N.Acc. afferent DA fibers might impose a modulatory influence on DA release within the N.Acc., and thus account for the naloxone-amphetamine interaction reported elsewhere [16,17] and herein. Opiate receptors located on cells within the N.Acc., however, might mediate the direct activating properties of opiate agonists such as heroin. While these pre- and post-synaptic influences are dissociable experimentally, it is likely that they function in concert in the intact organism.

ACKNOWLEDGEMENTS

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REFERENCES

1. Amalric, M. and G. F. Koob. Low doses of methylnaloxonium in the nucleus accumbens antagonize hyperactivity induced by heroin in the rat. *Pharmacol Biochem Behav* **23**: 411–415, 1985.
2. Atweh, S. and M. Kuhar. Autoradiographic localization of opiate receptors in the rat brain. III. The telencephalon. *Brain Res* **134**: 393–405, 1977.
3. Cuello, A. C. and G. Paxinos. Evidence for a long Leu-enkephalin striopallidal pathway in rat brain. *Nature* **271**: 178–180, 1978.
4. Elde, R., T. Hokfelt, O. Johansson and L. Terenius. Immunohistochemical studies using antibodies to leucine-enkephalin: initial observations on the nervous system of the rat. *Neuroscience* **1**: 349–355, 1976.
5. Farnebo, L. O. Effect of d-amphetamine on spontaneous and stimulation-induced release of catecholamines. *Acta Physiol Scand* **371**: 45–52, 1971.
6. Joyce, E. M. and G. F. Koob. Amphetamine-, scopolamine- and caffeine-induced locomotor activity following 6-hydroxydopamine lesions of the mesolimbic dopamine systems. *Psychopharmacology (Berlin)* **73**: 311–313, 1981.
7. Joyce, E. M., G. F. Koob, R. Strecker, S. D. Iversen and F. E. Bloom. The behavioral effects of enkephalin analogues injected into the ventral tegmental area and globus pallidus. *Brain Res* **221**: 359–370, 1981.
8. Kehne, J. H., W. W. Sant and C. A Sorenson. The effects of radio-frequency lesions of the nucleus accumbens on d-amphetamine-induced locomotor and rearing behavior in rats. *Psychopharmacology (Berlin)* **75**: 363–367, 1981.
9. Kelly, P. H. and S. D. Iversen. Amphetamine and apomorphine responses in the rat following 6OHDA lesions of the nucleus accumbens septi and corpus striatum. *Brain Res* **94**: 507–522, 1975.
10. Koob, G. F., L. Stinus and M. Le Moal. Hyperactivity and hypoactivity produced by lesions to the mesolimbic dopamine system. *Behav Brain Res* **3**: 341–359, 1981.
11. Mogenson, G. J. and M. A. Nielson. Evidence than an accumbens to sub-pallidal GABAergic projection contributes to locomotor activity. *Brain Res Bull* **11**: 309–314, 1982.
12. Pollard, H., C. Llorsen, J. Bonnet, J. Costentin and J. C. Schwartz. Opiate receptors on mesolimbic dopaminergic neurons. *Neurosci Lett* **7**: 295–301, 1977.
13. Rubin, A., H. Yen and M. Pfeffer. Psychopharmacological profile of molondone. *Nature* **216**: 578, 1967.
14. Schaefer, G. J. and R. P. Michael. Drug interactions on spontaneous locomotor activity in rats: neuroleptics and amphetamine-induced hyperactivity. *Neuropharmacology* **23**: 909–914, 1984.
15. Schwartz, J. T., H. Pollard, C. Llorens, B. Malfroy, C. Gross, P. L. Pradelles and F. Dray. Endorphins and endorphin receptors in striatum: relationships with dopaminergic neurons. In: *The Endorphins*, edited by E. Costa and M. Trabucci. New York: Raven Press, 1978, pp. 245–264.
16. Segal, D. S., R. G. Browne, A. Arnsten, D. C. Derrington, F. E. Bloom, R. Guillemin and N. Ling. Characteristics of β-endorphin induced behavioral activation and immobilization. In: *Endorphins in Mental Health Research*, edited by E. Usdin, W. E. Bunney and N. Kline. London: MacMillan, 1979, p. 307.
17. Swerdlow, N. R., F. J. Vaccarino and G. F. Koob. Effects of naloxone on heroin-, amphetamine-, and caffeine-stimulated locomotor activity in the rat. *Pharmacol Biochem Behav* **23**: 499–501, 1985.
18. Uhl, G. R., R. R. Goodman, M. J. Kuhar and S. H. Snyder. Enkephalin and neurotensin: immunohistochemical localization and identification of an amygdalofugal pathway. In: *Advances in Biochemistry and Psychopharmacology*, edited by E. Costa and M. Trabucci. New York: Raven Press, 1978, pp. 71–87.
19. Vaccarino, F. J., M. Amalric, N. R. Swerdlow and G. F. Koob. Blockade of amphetamine but not opiate-induced locomotion following antagonism of dopamine function in the rat. *Pharmacol Biochem Behav* **24**: 61–65, 1986.
20. Vaccarino, F. J., F. E. Bloom and G. F. Koob. Blockade of nucleus accumbens opiate receptors attenuates intravenous heroin reward in the rat. *Psychopharmacology (Berlin)* **86**: 37–42, 1985.
21. Van der Kooy, D., N. R. Swerdlow and G. F. Koob. Paradoxical reinforcing properties of apomorphine: effects of nucleus accumbens and area postrema lesions. *Brain Res* **259**: 111–118, 1983.
22. Wamsley, J. K., S. W. Young and M. J. Kuhar. Immunohistochemical localization of enkephalin in rat forebrain. *Brain Res* **190**: 153–174, 1980.
23. Zaborsky, L., G. F. Alheid, V. E. Alones, W. H. Oertel, D. E. Schmechel and L. Heimer. Afferents of the ventral pallidum studied with a combined immunohistochemical-anterograde degeneration method. *Soc Neurosci Abstr* **8**: 218, 1982.