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Aminergic Regulation of Release of Crustacean Hyperglycemic Hormone Isoforms and Blood Glucose Level

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ABSTRACT

Injection of dopamine into Procambarus clarkii resulted in significant hyperglycemic responses. Injection of A77636 (a D_1 receptor agonist) or quinpirole (a D_2 receptor agonist) also elicited significant hyperglycemic responses. Receptor antagonists were used to determine if these drugs were capable of blocking agonist-induced hyperglycemia. SCH23390 (a D_1 receptor antagonist) was unable to block A77636-evoked hyperglycemia, whereas eticlopride (a D_2 receptor antagonist) partly blocked the dopamine- or quinpirole-evoked hyperglycemia. Furthermore, pretreating the animals with eticlopride did not block the hyperglycemic effect of serotonin (5-HT), an amine neuroregulator also known to elicit hyperglycemic responses . Likewise, pretreating the animals with ketanserin or methysergide (both 5-HT receptor antagonist able to block 5-HT-induced hyperglycemia or CHH release) did not block the hyperglycemic effect of dopamine. Finally, in vivo injection of dopamine significantly increased hemolymph CHH levels. The combined results suggest that 1) dopamine is a potent hyperglycemic factor that increases the glucose levels, at least partly, by enhancing CHH release, 2) hyperglycemic effect of dopamine is mediated by D_1- and D_2-like receptors that are distantly related to vertebrate counterparts, and 3) 5-HTergic and dopaminergic are independent pathways in regulating glucose levels in Procambarus clarkii.

BACKGROUND

The X-organ/sinus gland complex located within the eyestalks of decapod crustaceans is an important endocrine system that produces and releases a host of regulatory neuropeptides (Cooke and Sullivan 1982; Keller 1992; Soyez 1997). Among these neuropeptides, crustacean hyperglycemic hormone (CHH) is involved in regulating blood glucose levels mainly through mobilization of glucose from the glycogen depots (see Santos and Keller 1993), although other regulatory functions have been proposed (e. g., Tensen et al. 1989; Charmantier-Daures et al. 1994; Yasuda et al. 1994; Liu et al. 1997). Sequence analysis of CHHs isolated from various decapods indicated that they are peptides of 72~73 amino acid residues with a considerable degree of homology (Soyez 1997). An intriguing feature of CHH is the existence of multiple molecular variants in a given species as has been reported in several species (Soyez et al. 1994, Yasuda et al. 1994; Aguilar et al. 1995; Soyez et al. 1997; Yang et al. 1997; Chung et al. 1998). Significance of this phenomenon of structural polymorphism remains unknown.

Basal and enhanced release of CHH in response to electrical and elevated [K^+] stimuli has been characterized

(Keller et al. 1994; Richmond et al. 1995). Furthermore, several neuroactive substances are implicated in the regulation of CHH release. It has been reported that in vivo injection of dopamine, enkephalin, or serotonin (5-hydroxytryptamine, 5-HT) altered blood glucose levels, and suggested that the glycemic response was due to increased or decreased CHH release (Keller and Beyer 1968; Fingerman et al. 1981; Jaros 1990; Rothe et al. 1991; Kuo et al. 1995; Sarojini et al. 1995; Lee et al. 2000a). However, it has never been confirmed that release of CHH is indeed altered in response to any of these neuroactive substances.The objectives of the present study were to elucidate the roles of aminergic neuronal pathways in regulating glucose levels and release of CHH. Specifically, we had 1) characterized the types of receptosr that mediate dopamine's hyperglycemic action, 2) confirmed that dopamine stimulates the release of CHH, and 3) determined that serotonergic and dopaminergic neurons represent independent inputs that regulate blood glucose levels.

MATERIALS AND METHODS

Experimental procedures

All experimental animals (Procambarus clarkii) were deprived of food 2 days prior to injection. For eyestalk ablation, animals were anaesthetized on ice and eyestalks removed using a pair of fine scissors also 2 days prior to injection. Tested pharmacological agents (see RESULTS) were dissolved in vehicle, either crayfish saline (Van Harreveld, 1936) buffered with 20 mM HEPES or DMSO (dimethyl sulfoxide, Sigma), and injected into animals through the base of the chelae with an insulin syringe coupled to a 29 gauge needle (Terumo Medical Corp., Elkton, MD, USA). In the experiments where the effects of receptor antagonists on 5-HT- or dopamine-induced hyperglycemia were examined, animals received an injection of the appropriate antagonist 10 min prior to an injection of 5-HT or dopamine; both injections were administered as described above. In all experiments, hemolymph was withdrawn from animals one hour (except where noted otherwise) after the last injection. Four parts of the hemolymph were diluted immediately with one part of saline containing 0.01 M EDTA to inhibit clotting, and the mixture was centrifuged (2,000Xg, 20 min, 4 ℃). The resultant supernatant was collected and immediately used for glucose analysis as described below.

Glucose analysis

Blood glucose was quantified using the Trinder Glucose Assay Kit (# 315; Sigma) according to the manufacturer's protocol. Briefly, the hemolymph preparation (25 μl) and the Trinder reagent (200 μl) were added to the wells of a microplate, and mixed using a microplate shaker. After standing at room temperature for 10 min, the optical

density was read using an ELISA reader (Elx 808 IU, Bio-Tek Instruments Inc., Winooski, VT, USA) at 490 nm. A standard curve was constructed for each microplate using glucose standards (# 16-100, Sigma). Glucose concentration in samples was inferred from the standard curve and corrected for the dilution factor using KC4 software from Bio-Tek Instruments Inc.

CHH ELISA

Hemolymph CHH levels in the animals subjected to dopamine injection were analyzed using a sandwich type ELISA. Specificity of the α-CHH serum used in the assay has been described elsewhere (Lee et al., submitted).

RESULTS

Injection of dopamine resulted in significant hyperglycemic responses in intact animals The effective doses range from 5×10^{-9} to 1×10^{-6} mol/animal (Fig. 1C-H), which elicited 2.1- to 7.2-fold increases. A time course of dopamine-induced hyperglycemia is shown in. The blood glucose levels increased significantly within 30 min of injection, reached a peak at 1 hr, then declined, reaching basal levels at 4 hr; saline-injected controls did not show changes in blood glucose levels over time (Fig. 2).

Dopamine-induced hyperglycemia was also observed in eyestalk-ablated animals. It was shown that dopamine (10^{-7} mol/animal) significantly increased glucose level (Fig. 3). The fold increase (~200% of saline-injected control) was, however, lower than that provoked in intact animals by the same dose of dopamine (see Fig. 1).

Agonists for vertebrate dopamine receptor also elicited hyperglycemic responses. Injection of A77636 (a D_1 receptor agonist) or quinpirole (a D_2 receptor agonist) elicited significant hyperglycemic responses (Fig. 4 and 5). A77636 was more potent than quinpirole in eliciting hyperglycemic responses. At the highest dose tested, the former evoked a 6.6-fold increase in glucose levels, whereas the latter a 3.7-fold increase. Receptor antagonists were also used to determine if these drugs are capable of blocking agonist-induced hyperglycemia. SCH23390 (a D_1 receptor antagonist) was unable to block A77636-evoked hyperglycemia. Paradoxically, SCH23390, at the highest dose tested, potentiated the effect of A77636 (Fig. 6); eticlopride (a D_2 receptor antagonist) partly blocked the dopamine- or quinpirole-evoked hyperglycemia (Fig. 7 and 8).

Previous studies conducted in our laboratory indicated that serotonin (5-HT) is another biogenic amine that is able to elicit hyperglycemic responses (Lee et al., 2000; Lee et al., submitted). Thus, we are interested in determining if 5-HTergic and dopaminergic neurons are sequential or independent pathways in elevating glucose levels. Pretreating the animals with eticlopride did not block the hyperglycemic effect of 5-HT (Fig. 9). Likewise, pretreating the animals with ketanserin or methysergide (both 5-HT receptor antagonist able to block 5-HT-induced hyperglycemia or CHH release. Lee et al., 2000; Lee et al., submitted) did not block the hyperglycemic effect of dopamine (Fig. 10).

Finally, it is determined that dopamine also stimulated the release of CHH. In vivo injection of dopamine significantly increased hemolymph and CHH levels (Fig. 11 and 12).

DISCUSSION

Several conclusions could be drawn from the results obtained in the present study. Dopamine acts as a potent hyperglycemic factor in the crayfish. Both intra- and extra-eyestalk sites are likely targets for dopamine's action. The identity of the extra-eyestalk sites remains to be determined. It is likely that dopamine directly acts on the glycogen depots (e.g., the hepatopancreas, the muscle, etc.) that release glucose in response. As to the intra-eyestalk site, it is most plausible that dopamine acts on the X-organ/sinus gland system, which releases CHH in response. In fact, our data showing that dopamine injection increased blood CHH levels directly support such proposition.

In direct contrast with our results, Sarojini et al. reported that dopamine decreased blood glucose levels presumably by inhibiting release of CHH in P clarkii (1995). The reason for such difference is unknown. However, it is noted that the injection volume used in that study was 100 µl, as opposed to 10 µl used in our study. Preliminary testing in our lab indicated that high vehicle volume (> 50 µl) itself significantly increased blood glucose levels. This explains the fact that the glucose level in their control (saline-injected) animals was ~ 35 mg/dL, whereas the corresponding value was ~ 10 mg/dL in the present study. Apparently, direct comparison of these two studies is impossible because the basal state was different between their and our animals. On the other hand, Kuo et al (1995) and Luschen et al (1993) reported that dopamine elicited hyperglycemic responses in Penaeus monodon and Carcinus maenas, respectively.

Receptors with which dopamine interacts in eliciting hyperglycemic responses were also characterized. Although both D_1 and D_2 receptor agonists were able to evoke

hyperglycemic responses, only D_2 (but not D_1) receptor antagonist partly blocked the agonist-induced hyperglycemia. The overall results indicated that the involved receptors are pharmacologically distinct from their vertebrate counterparts, a conclusion also reached by investigators working on other invertebrate systems. Kuo et al also concluded that the hyperglycemic responses to dopamine in P. monodon are mediated by a D_1 (but not D_2) receptor (1995). Their conclusion was based on the results that the dopamine-evoked hyperglycemia was mimicked by a D_1 receptor agonist (SKF 38393), and blocked by a D_1 receptor antagonist (SCH23390). Thus, dopamine receptors involved in regulating blood glucose levels appear species-specific.

It is known from several crustacean studies that stressors of various natures (extreme temperature, hypoxia, organic and inorganic pollutants, bacterial infection, etc.) induce hyperglycemia and/or CHH release (Fingerman et al. 1981; Reddy et al. 1994; Webster 1996; Lorenzon et al. 1997; Chang et al. 1998; the present study). However, the neural network conveying these environmental stress signals remain undefined. It would be interesting to determine in future studies if stress-evoked hyperglycemia is mediated by dopamine-induced CHH release.

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FIGURE LEGENDS

Fig. 1. Dose-dependent effect of dopamine (DA) on the blood glucose levels in intact *P. clarkii*. One hour after injection of vehicle (A) or vehicle containing different doses (B-H) of dopamine, hemolymph was withdrawn from animals for glucose quantification. Doses of dopamine : B, 1×10^{-9} : C, 5×10^{-9} : D. 1×10^{-8} : E, 5×10^{-8} : F, 1×10^{-7} : G, 5×10^{-7} : H, 1×10^{-6} (mol/animal). Each column represents a mean ± SEM (n = 8). * and ** represent significant difference from saline control at 5% and 1% levels, respectively.

Fig. 2. Time course of dopamine-induced hyperglycemia. After injection of saline containing dopamine (10^{-7} mol/animal), hemolymph was withdrawn from animals at the time points indicated for glucose quantification. To avoid repeated bleeding, a separate group of animals was used for each time point. Each point represents a mean ± SEM (n = 8). * and ** represent significant difference from zero time control at 5% and 1% levels, respectively.

Fig. 3. Dependency of dopamine-induced hyperglycemia on the presence of eyestalks. Eyestalk-ablated animals received an injection of saline or saline containing dopamine (10^{-7} mol/animal). Each column represents a mean ± SEM (n = 8). * represents significant difference from saline at 5% levels.

Fig. 4. Dose-dependent effect of selective D_1 receptor agonist on the blood glucose levels in intact *P. clarkii*. One hour after injection of vehicle (A) or vehicle containing different doses (B-E) of A77636, hemolymph was withdrawn from animals for glucose quantification. Doses of A77636 : B, 1×10^{-9} : C, 1×10^{-8} : D, 1×10^{-7} : E, 1×10^{-6} (mol/animal). Each column represents a mean ± SEM (n = 8). * and ** represent significant difference from saline control at 5% and 1% levels, respectively.

Fig. 5. Dose-dependent effect of selective D_2 receptor agonist, on the blood glucose levels in intact *P. clarkii*. One hour after injection of vehicle (A) or vehicle containing different doses (B-G) of quinpirole, hemolymph was withdrawn from intact animals for glucose quantification. Doses of quinpirole : B, 1×10^{-8} : C, 5×10^{-8} : D, 1×10^{-7} : E, 2.5×10^{-7} : F, 5×10^{-7} : G, 1×10^{-6} (mol/animal). Each column represents a mean ± SEM (n = 8). * and ** represent significant difference from saline control at 5% and 1 % levels, respectively.

Fig. 6. Effects of selective D_1 receptor antagonist on A77636-induced hyperglycemia. Intact animals were pretreated with an injection of vehicle (A-B) or vehicle containing different doses (C-F) of SCH23390 10 min before receiving an injection of saline or saline containing a subsaturating dose (10^{-7} mol/animal) of A77636. Hemolymph was withdrawn from animals for glucose quantification. Doses of SCH23390 : C, 1×10^{-9} : D, 1×10^{-8} : E, 1×10^{-7} : F, 1×10^{-6} (mol/animal). Each column represents a mean ± SEM (n = 8). * represents significant difference from column B at 5% levels.

Fig. 7. Effects of selective D_2 receptor antagonist on dopamine-induced hyperglycemia. Intact animals were pretreated with an injection of vehicle (A-B) or vehicle containing different doses (C-E) of eticlopride 10 min before receiving an injection of saline or saline containing a subsaturating dose (5×10^{-7} mol/animal) of dopamine (DA). Hemolymph was withdrawn from animals for glucose quantification. Doses of eticlopride : C, 1×10^{-9} : D, 1×10^{-8} : E, $1 \times$

10^{-7} (mol/animal). Each column represents a mean ± SEM (n = 8). * represents significant difference from column B at 5% levels.

Fig. 8. Effects of selective D_2 receptor antagonist on quinpirole-induced hyperglycemia. Intact animals were pretreated with an injection of vehicle (A-B) or vehicle containing different doses (C-F) of eticlopride 10 min before receiving an injection of saline or saline containing a subsaturating dose (5×10^{-7} mol/animal) of quinpirole. Hemolymph was withdrawn from animals for glucose quantification. Doses of Eticlopride : C, 1×10^{-9} ; D, 1×10^{-8} ; E, 1×10^{-7} ; F, 1×10^{-6} (mol/animal). Each column represents a mean ± SEM (n = 8). * represents significant difference from column B at 5% levels.

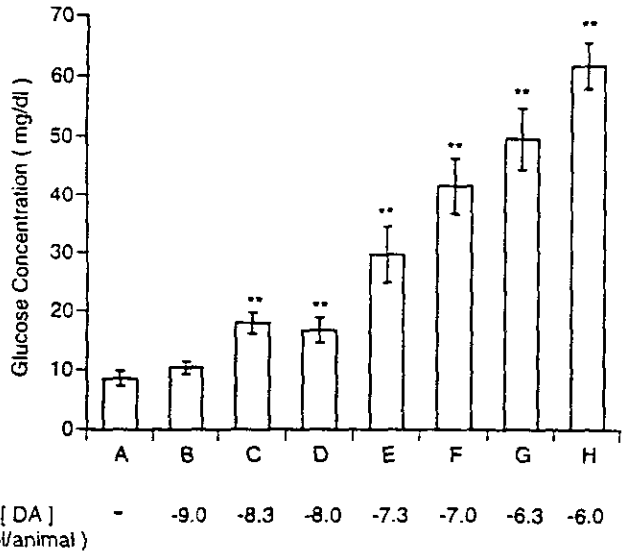
Fig. 9. Effects of selective 5-HT_{2A} receptor antagonist on dopamine-induced hyperglycemia. Intact animals were pretreated with an injection of 10 % DMSO (A-B) or 10% DMSO containing different doses (C-D) of ketanserin 10 min before receiving an injection of saline or saline containing a subsaturating dose (5×10^{-7} mol/animal) of dopamine (DA). Hemolymph was withdrawn from intact animals for glucose quantification. Doses of Ketanserin : C, 1×10^{-8} ; D, 1×10^{-7} (mol/animal). Each column represents a mean ± SEM (n = 8).

Fig. 10. Effects of selective 5-$HT_{1/2}$ receptor antagonist on dopamine-induced hyperglycemia. Intact animals were pretreated with an injection of vehicle (A-B) or vehicle containing different doses (C-D) of methysergide 10 min before receiving an injection of saline or saline containing a subsaturating dose (5×10^{-7} mol/animal) of dopamine (DA). Hemolymph was withdrawn from animals for glucose quantification. Doses of methysergide : C, 1×10^{-8} ; D, 1×10^{-7} (mol/animal). Each column represents a mean ± SEM (n = 8).

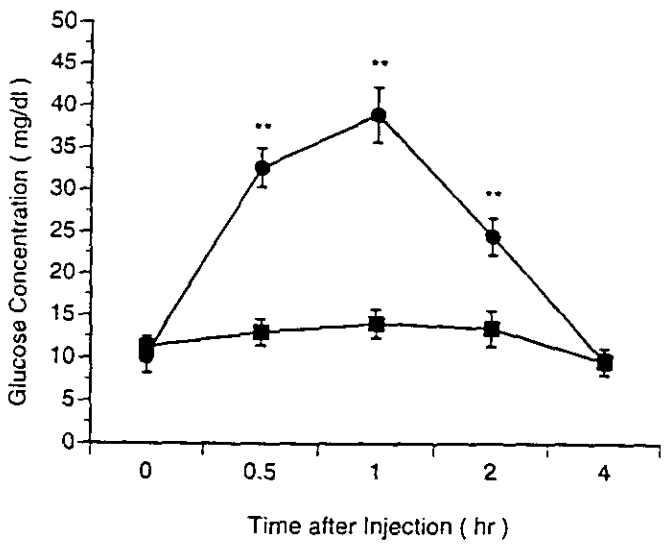
Fig. 11. Effect of dopamine on the blood glucose levels in intact *P. clarkii*. One hour after injection of saline containing a subsaturating dose (5×10^{-7} mol/animal) of dopamine, hemolymph was withdrawn from animals for glucose quantification. Each column represents a mean ± SEM (n = 8). ** represents significant difference from basal control at 1% levels.

Fig. 12. Effect of dopamine on the blood CHH levels in intact *P. clarkii*. One hour after injection of saline containing a subsaturating dose (5×10^{-7} mol/animal) of dopamine, hemolymph was withdrawn from animals for CHH quantification. Each column represents a mean ± SEM (n = 8). ** represents significant difference from basal control at 1% levels.

1.


Glucose Concentration (mg/dl)
70
60
50
40
30
20
10
0
A B C D E F G H
Log [DA] - -9.0 -8.3 -8.0 -7.3 -7.0 -6.3 -6.0
(mol/animal)

2.


Glucose Concentration (mg/dl)
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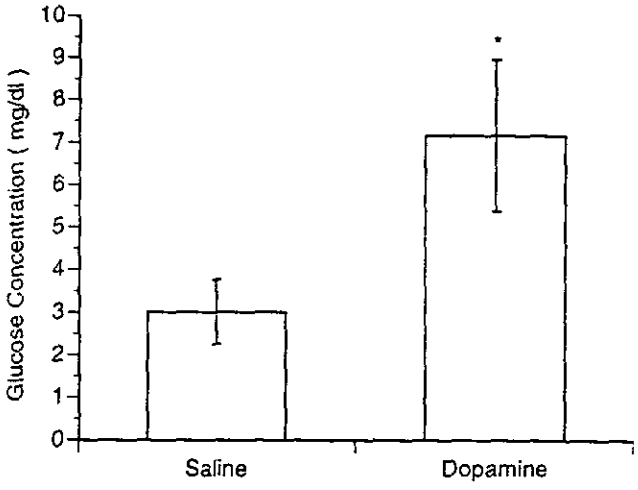
45
40
35
30
25
20
15
10
5
0
0 0.5 1 2 4
Time after Injection (hr)

3.


Glucose Concentration (mg/dl)
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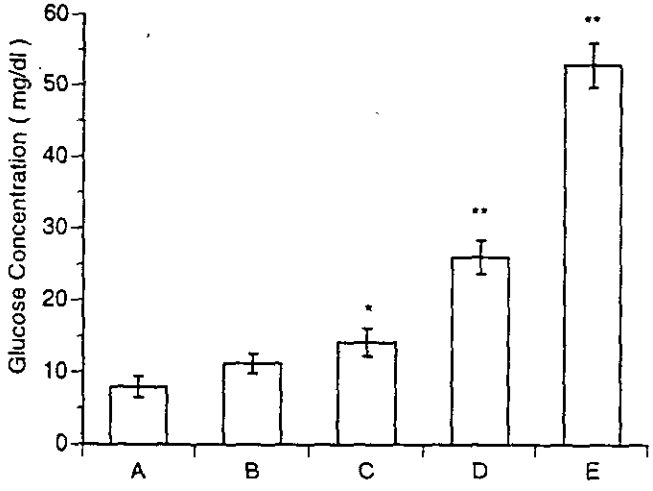
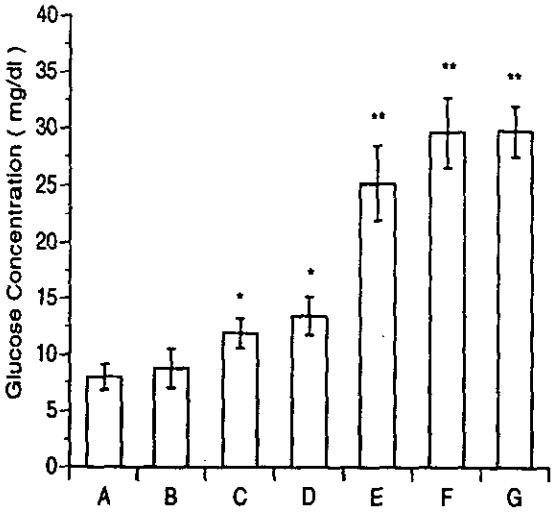
9
8
7
6
5
4
3
2
1
0
Saline
Dopamine

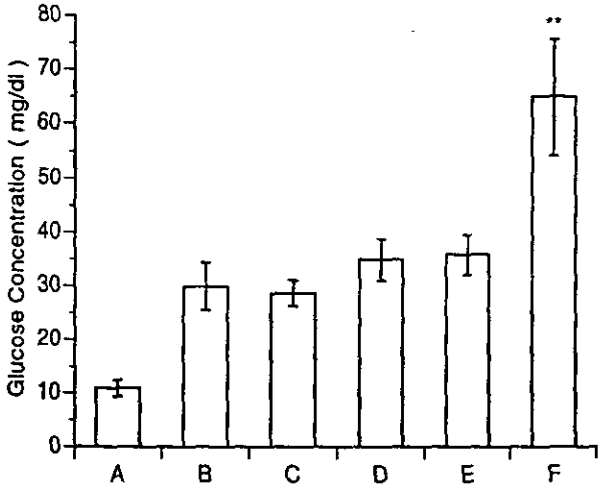
4.


Glucose Concentration (mg/dl)
60
50
40
30
20
10
0
A B C D E
Log [A77636] - -9 -8 -7 -6
(mol/animal)

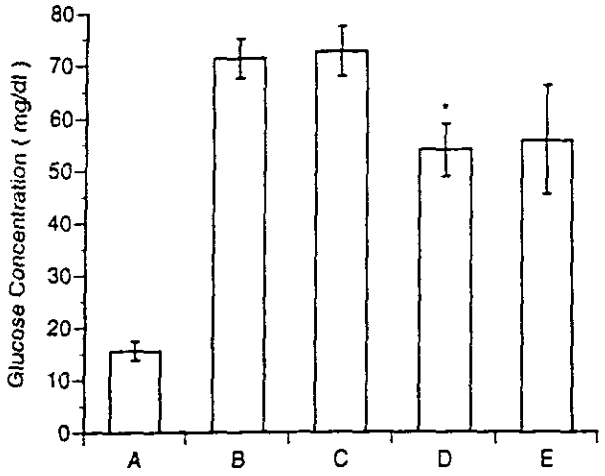
5.


Glucose Concentration (mg/dl)
40
35
30
25
20
15
10
5
0
A B C D E F G
.og [Quinpirole] - -8.0 -7.3 -7.0 -6.6 -6.3 -6.0
mol/animal)

6.

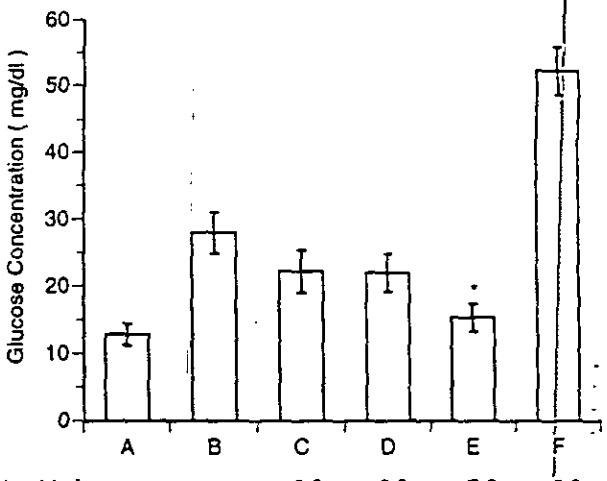

Glucose Concentration (mg/dl)
80
70
60
50
40
30
20
10
0
A B C D E F
Log [SCH 23390] - -9 -8 -7 -6
Log [A77636] -7 -7 -7 -7 -7
(mol/animal)

7.



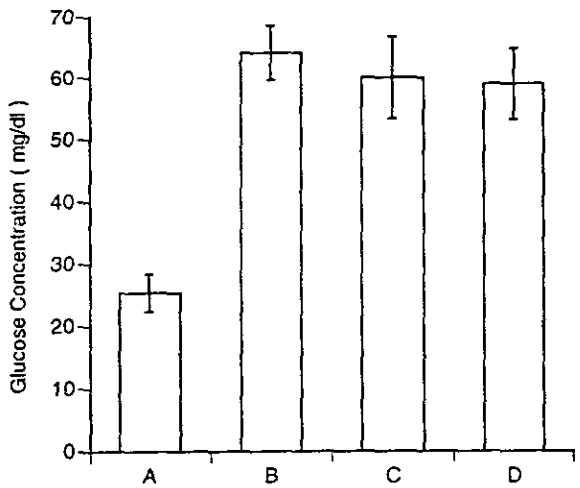
Log [Eticlopride]	-	-9.0	-8.0	-7.0
Log [DA]	-6.3	-6.3	-6.3	-6.3
(mol/animal)				

8.



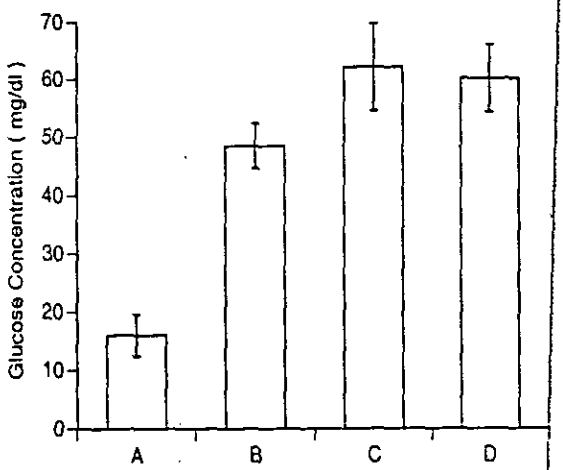
Log [Eticlopride]	-	-9.0	-8.0	-7.0	-6.0
Log [Quinpirole]	-6.3	-6.3	-6.3	-6.3	-6.3
(mol/animal)					

9.



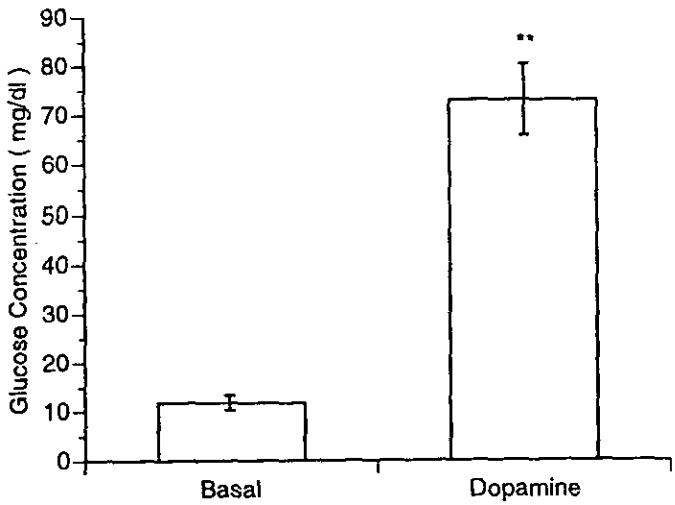
Log [Ketanserin]	-	-8.0	-7.0
Log [DA]	-6.3	-6.3	-6.3
(mol/animal)			

10.



Log [Methysergide]	-	-8.0	-7.0
Log [DA]	-6.3	-6.3	-6.3
(mol/animal)			

11.



12.