Pharmacology Biochemistry & Behavior, Vol. 19, pp. 957–961, 1983. © Ankho International Inc. Printed in the U.S.A.

Behavioral Comparison of Pentylenetetrazol, Clonidine, Chlordiazepoxide and Diazepam in Infant Rats

BRUCE A. PAPPAS AND PETER WALSH

Unit for Behavioral Medicine and Pharmacology, Department of Psychology
Carleton University, Ottawa, Ontario, K1S 5B6

Received 14 December 1982

PAPPAS, B. A. AND P. WALSH. *Behavioral comparison of pentylenetetrazol, clonidine, chlordiazepoxide and diazepam in infant rats.* PHARMACOL BIOCHEM BEHAV **19**(6) 957–961, 1983.—The effects of various doses of pentylenetetrazol, clonidine, chlordiazepoxide and diazepam on limb and head movement and behavioral seizure signs were examined in 4- , 8- and 16-day old rats tested at ambient temperatures of either 25 or 35°C. All 4 drugs produced intense behavioral activation at the 2 younger ages but there were marked differences among them in the effects of test temperature on this activation and in the relationship between age and their activating effect. A "paradoxical" and intense behavioral energization was observed after the administration of either of the 2 benzodiazepines at 4, 8 but not 16 days, particularly at the lower test temperature. Clonidine and pentylenetetrazol were activating at all 3 ages but while clonidine had greater effect at the low test temperature, the opposite was the case after pentylenetetrazol. The effects of the benzodiazepines and clonidine were clearly distinct from those of pentylenetetrazol and this was the only drug to substantially elicit seizure signs. It is uncertain whether or not the benzodiazepines cause brain seizures in young animals. If so, then their behavioral manifestation is clearly different from that observed after pentylenetetrazol.

Pentylenetetrazol Clonidine Benzodiazepines Seizures Behavior Infant rats

PREVIOUS reports suggest an unexpected similarity in the behavioral effects of benzodiazepines, clonidine and pentylenetetrazol in rat pups. For example, this laboratory has reported that diazepam elicited very active behavior in rats up to nine days of age, characterized by swimming movements of the limbs and intense forward crawling even when the latter was impeded by the walls of the test apparatus (Pusztay *et al.* [11]. Our (unpublished) studies also indicated a similar effect after the hypnotic benzodiazepine flurazepam. At 12 days and older, we have found that these benzodiazepines either had no effect or inhibited activity. Our observations were strikingly similar to those reported for clonidine which activates rats until about 20 days of age after which it has an apparent sedating effect [9, 12]. The nature of this activating effect of clonidine in young rats was described as "forward locomotion, paddling and wallclimbing" by both Nomura and Segawa ([9] pp. 532) and Reinstein and Isaacson ([13] pp. 379). Our data for the benzodiazepines and those of others for clonidine did indicate however that their activating effects disappeared at different ages, namely after about 9 and 20 days respectively.

We have also included here a detailed analysis of the chemical convulsant pentylenetetrazol. As first described by Millichap [7] and later elaborated by Vernadakis and Woodbury [15], there is a distinctive maturation of seizure characteristics caused by this drug (and to maximal electroshock procedure). Up to eight days of age the rat displays "hyperkinesia (paddling and running movements, shaking and hyperextension of the head)" ([15] pp. 165). These "paddling and running movements" seem similar to those observed after clonidine and up to about 9 days, after the benzodiazepines. Furthermore, these locomotor activating effects of pentylenetetrazol reportedly disappear at the same age at which we observed a disappearance of the activating effects of the benzodiazepines. Thus convulsant (pentylenetetrazol) and anti-convulsant (benzodiazepine) agents reportedly elicit some similar effects in young rats as well as a parallel maturational time course for these effects. Does this reflect seizure induction in neonatal rats, by the benzodiazepines? While this seemed an unlikely possibility insofar as the benzodiazepines are potent anticonvulsants in the adult, Barr and Lithgow [1] recently reported that both chlordiazepoxide and flurazepam when administered at typical antiseizure doses, produced "behavioral convulsions" (pp. 431) in rats 18 days of age or younger. In 3 and 10 day old rats these "convulsions" were characterized by "swimming" movements. In their experiment however, these benzodiazepines were not compared with a typical

convulsant drug thus raising the possibility that what these experimenters observed were not typical manifestations of brain seizure activity.

In this study we compared the effects of various doses of chlordiazepoxide, diazepam, clonidine and pentylenetetrazol using an observational sampling procedure to score the presence of limb movements, head movements and seizure signs. In view of the facts that ambient temperature affects dopaminergic brain function in young rats (Horwitz *et al.* [6]) and that their activity is modulated by dopaminergic mechanisms (Pappas *et al.* [10]), we assessed the effects of these drugs at ambient temperatures near (35°C) or below (25°C) litter temperature.

METHOD

Subjects

A total of 547 offspring of Wistar rats, purchased 14 days pregnant from Woodlyn Farms (Guelph, Ontario), were used. Upon receipt from the supplier the dams were housed in polystyrene maternity cages. The day of birth was designated as day one of life. A cycle of lights on at 0700 and off at 2000 hours was maintained.

Apparatus

Four empty polystyrene cages (25×15×12 cm) served as observation containers. The air temperature of these containers was maintained at either 25°C or 35°C with two General Electric 250 watt, infrared lamps which were activated by a proportional temperature controller (YSI model 72).

Drugs

The drugs and their doses used in the study were clonidine HCl (Boehringer, Ingelheim) 100, 300 and 900 μg/kg, pentylenetetrazol (Sigma) 25, 50 and 75 mg/kg, chlordiazepoxide (Hoffman, La Roche) 1.0, 3.0 and 9.0 mg/kg and diazepam (Hoffman, La Roche) 0.5, 1.5 and 4.5 mg/kg. The vehicle used for the first three drugs was normal saline but for the latter another vehicle was necessary. It consisted of 1.5% benzyl alcohol, 40.0% propylene glycol, 10.0% ethanol, 2.5% sodium benzoate and 2.5% benzoic acid (buffers), 43.5% distilled H_2O, and its pH was adjusted to 6.7 using sodium hydroxide.

Procedure

At the respective testing ages of 4, 8 or 16 days, four rat pups, usually from the same litter, were each placed randomly into one of the four clean observation containers. Using a thermistor probe which was centered on the floor equidistant from the four containers, the temperature at this level was preset to be at either 25°C or 35°C (±1°C) where it remained during the testing procedure. After each animal was weighed, the amount of injection was determined. This process took approximately ten minutes and allowed the pups to adapt to the new environment. Drug, drug dose and vehicle injections were assigned to each container in a semirandom fashion prior to testing. Recording began after injections had been given subcutaneously in the nape of the neck. Behavior was recorded over a 60 minute period for each animal as described below. The animals were then immediately sacificed by overdose of pentobarbital. At each of the two temperatures, seven pups of either 4, 8 or 16 days of age received either a vehicle injection or one of three doses of the four drugs. The data for one animal were lost (chlordiazepoxide 3 mg/kg at four days at 25°C) while one additional rat was inadvertently run in two other groups (chlordiazepoxide 1 mg/kg at 8 days and chlordiazepoxide 9 mg/kg at 4 days, both at 25°C).

Behavioral Scoring

Behavioral scoring was done using a time sampling procedure and by the same experimenter for the entire experiment. Behavior was observed and recorded for 15 seconds for each of the 4 animals. This procedure was repeated every minute for 60 minutes. The presence or absence of 13 behaviors was scored and these were then categorized a priori into limb movement (LM: consisting of forelimb or hindlimb movement, single limb jerks, crawling, swimming-like movements without body locomotion, walking, rearing, wall progression), head movement (HM: head turning, head raising) and seizure-like activity (head-shaking, forelimb clonus, hindlimb clonus, body clonus, see Vernadakis and Woodbury, [15]). Rats were also scored for the absence of body righting and for vocalizations. These data were examined separately since there was obvious a priori categorization for them.

For statistical analyses, the total number of observations of the behaviors were summed for the 60 observation periods so as to yield three scores for each rat (i.e. total scores for LM, HM and seizures).

RESULTS

Limb Movement (LM)

Vehicle (VEH). The two vehicle groups were compared with an analysis of variance. They did not differ, $F(2,74)=0.92$, NS, and were collapsed to form a single VEH control group for Fig. 1 and for further statistical analyses. As shown by Fig. 1, LM in these control rats was very much determined by temperature at 4 and 8 but not 16 days. At 4 and 8 days the rats showed higher LM at 35° than at 25°C. Analysis of variance indicated both a significant temperature, $F(1,74)=19.17$, $p<0.001$, and temperature by age interaction, $F(2,74)=17.23$, $p<0.001$.

Pentylenetetrazol (PTZ). Most rats receiving 75 mg/kg died and consequently this group was eliminated early in the experiment. At the lowest dose (25 mg/kg), the results for PTZ were not different than those for VEH. Thus at 4 and 8 days the rats showed higher LM at 35° than at 25°C. Analysis of variance incorporating the VEH and the two PTZ dose groups showed a significant dose main effect, $F(2,152)=41.00$, $p<0.001$, with LM highest after 50 mg/kg PTZ. This analysis also indicated no significant temperature by dose interaction, $F(2,152)=0.64$, N.S. As with vehicle pups, after both doses of PTZ there was greater LM at 35°C for 4 and 8 but not 16 day old pups. There was also a significant age by dose interaction, $F(4,152)=3.72$, $p<0.01$, due to the fact that with higher PTZ dose there was a high level of LM at 16 days. With both the lower PTZ dose and vehicle, LM declined from eight to 16 days.

Clonidine (CLON). Clonidine clearly elevated LM, $F(3,188)=84.80$, $p<0.001$, with this effect somewhat larger at 300 μg/kg than the other two doses. The most striking effect was a reversal of the temperature effect that was observed after VEH and PTZ. LM was significantly, $F(1,188)=67.43$, $p<0.001$, higher at 25°C for all three CLON doses. Generally, CLON enhanced LM at all three ages.

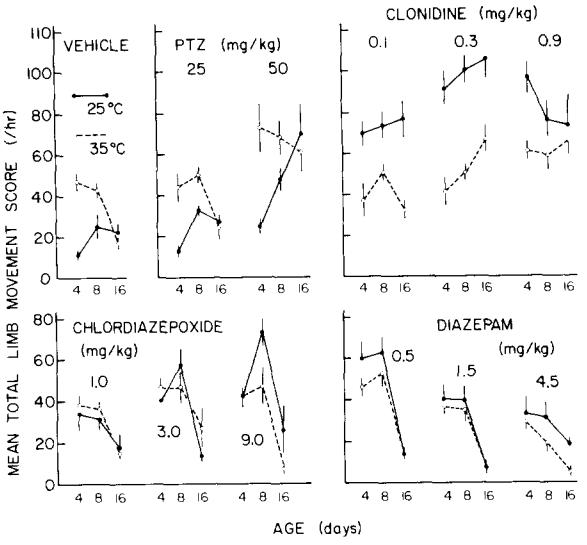

FIG. 1. The effects of vehicle, pentylenetetrazol (PTZ), clonidine, chlordiazepoxide and diazepam on mean (±s.e.) total limb movement (LM) scores at 4, 8 and 16 days of age. Each drug is represented in separate panels, one for each dose where applicable. The doses are indicated along with the drug in each panel. The ages are represented on the abscissa. Solid lines represent curves for rats run at 25°C ambient temperature while dashed lines give the curves for rats run at 35°C.

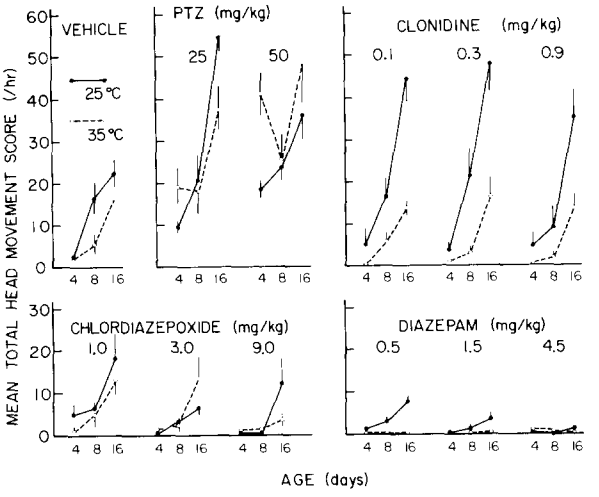

FIG. 2. The effects of vehicle, pentylenetetrazol (PTZ), clonidine, chlordiazepoxide and diazepam on mean (±s.e.) total head movement (HM) scores at 4, 8 and 16 days of age. Each drug is represented in separate panels, one for each dose where applicable. The doses are indicated along with the drug in each panel. The ages are represented on the abscissa. Solid lines represent curves for rats run at 25°C ambient temperature while dashed lines give the curves for rats run at 35°C.

Chlordiazepoxide (CDZ). As was observed from CLON, CDZ's effects on LM were temperature and dose dependent, as indicated by the significant interaction between these variables, $F(3,189)=821$, $p<0.001$. At the 1.0 and 3.0 mg/kg doses, there was minimal effect of temperature on LM. Unlike the VEH rats tested at four and eight days, after 9.0 mg/kg of CDZ the pups were more active at 25°C. Thus as with CLON but less dramatically so, CDZ eliminated or reversed the relationship between temperature and LM that had been shown by vehicle treated pups. Furthermore CDZ at the lower but not the higher temperature, elevated LM at 4 and 8 days, whereas at 16 days it had no obvious effect.

Diazepam (DZP). Like CLON and CDZ, diazepam reversed the relationship between temperature and LM which was shown by vehicle injected rats, $F(3,188)=8.77$, $p<0.001$. The DZP treated rats showed higher LM at 25°C. There was also a clear relationship between dose and LM, $F(3,188)=15.40$, $p<0.001$. The lowest dose (0.5 mg/kg) elevated LM at 25°C while the two higher doses suppressed LM at 35°C. This was reflected by a significant temperature by dose interaction, $F(3,188)=8.77$, $p<0.001$. Finally like CDZ, DZP at 0.5 and to some extent at 1.5 mg/kg elevated activity in 4 and 8 day old pups but failed to affect or slightly suppressed that of 16 day old pups. As was found for CDZ, this elevation at 4 and 8 was temperature dependent and occurred only in pups tested at 25°C.

Head Movement (HM)

Vehicle. There was no difference in HM between the two vehicle groups, $F(1,74)=0.04$, N.S., and they were combined for statistical purposes into one VEH group. As Fig. 2 shows, HM increased with age, $F(2,74)=23.63$, $p<0.001$. More HM was evident at 25°C for 8 and 16 day old pups. The age by temperature interaction fell short of statistical significance, however, $F(2,74)=2.30$, $p<0.11$.

Pentylenetetrazol. Both doses of PTZ elevated HM, $F(2,152)=47.82$, $p<0.001$. At the lower dose of PTZ, there was no effect of temperature. However, at the higher dose HM was somewhat greater for rats tested at 35°C. As was found for the VEH pups, HM was highest at 16 days for the PTZ rats.

Clonidine. Clonidine elevated HM, $F(3,188)=3.02$, $p<0.05$, but only at 25°C as indicated by the significant effect of temperature, $F(1,188)=74.70$, $p<0.001$, and temperature by drug dose interaction, $F(3,188)=4.38$, $p<0.01$. This is similar to the results for LM where CLON was also activating only at the lower temperature. Like after VEH and PTZ, CLON treated pups showed highest HM at 16 days of age.

Chlordiazepoxide. Generally CDZ suppressed HM in a dose dependent manner, $F(3,189)=10.28$, $p<0.001$. The temperature effect for these rats fell just short of significance, $F(1,189)=3.66$, $p<0.06$, and as Fig. 2 shows, there was no obvious relationship. As was found for VEH, PTZ and CLON, the greatest HM was seen at 16 days for the CDZ treated rats.

Diazepam. DIAZ suppressed HM in a dose dependent fashion at 25°C, $F(3,188)=34.64$, $p<0.001$, and abolished HM at 35°C with all doses. In this respect it resembled CDZ although Fig. 2. shows it was clearly more potent.

Seizure Scores

The seizure scores were collapsed across temperature since

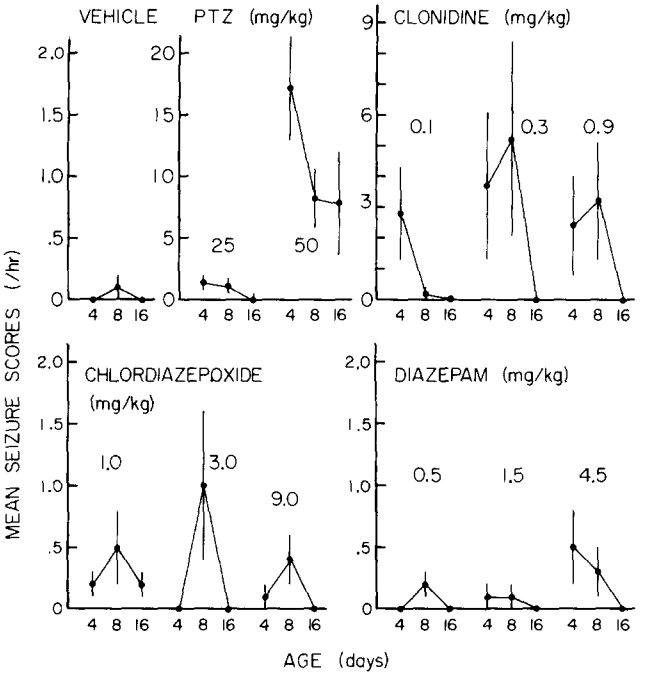

FIG. 3. The effects of vehicle, pentylenetetrazol (PTZ), clonidine, chlordiazepoxide and diazepam on mean seizure scores. Each drug is represented in separate panels, one for each dose where applicable. The age of testing is represented on the abscissa. Standard errors are shown only when they exceed the width of the symbol. Note that the scale of the vertical axes varies.

this variable had no discernible effect, and are presented in Fig. 3. The rats who had been injected with 50 mg/kg PTZ were clearly differentiated from the other drug and VEH groups by their high incidence of seizure signs. Seizure scores for this group declined with age, a finding which is consistent with the fact that the ED50 for PTZ markedly increases over the first few weeks of life [15]. As this figure shows, neither CDZ nor DZP elevated seizure scores at any age and dose combination. CLON elicited seizure signs in some 4- and 8-day rats but their incidence was considerably less than that after PTZ and did not significantly differ from the incidence observed for the VEH group.

Other Observations

The absence of the righting reflex was also scored for each rat. Loss of this reflex was considered an indicator of "behavioral convulsion" by Barr and Lithgow [1] and shown by them to be a characteristic BZP effect in immature rats. We found the righting reflex to be markedly and equally reduced by CLON and DIAZ, less so by CDZ and not at all (in comparison to VEH) by PTZ. Conversely, vocalizations were obviously increased by CLON and PTZ but unaffected by the benzodiazepines.

DISCUSSION

The impetus for this experiment arose from published observations concerning the effects of PTZ and CLON and our own observations with CDZ and DZP suggesting that these drugs had similar activating effects on rat pups. Further, since the completion of the present experiment, Barr and Lithgow [1] have reported that CDZ and flurazepam caused intense locomotor activation in infant rats which they interpreted as reflecting "behavioral convulsions." The present experiment confirmed at least one aspect of these earlier studies insofar as we found that at least at one dose, all of these drugs were behaviorally activating. The similarities ended there however, and there were some striking differences. Most noteworthy perhaps, is that the effects of the 2 benzodiazepines were clearly distinguishable from those of the chemical convulsant PTZ. Thus we question whether the benzodiazepine's in fact induce brain seizures in young rats as suggested by Barr and Lithgow [1].

One obvious difference was the divergent effects of test temperature on the limb activating effects of PTZ (and VEH) and the other 3 drugs. In aggreement with earlier work (Moorcroft *et al.* [8], limb activity was higher at 35°C than at 25°C for the 4 and 8 day old VEH rats. PTZ injected rats also showed more limb movement at the higher temperature. With CLON, CDZ and DZP there was a striking reversal of this relationship. The younger rats who were injected with these 3 drugs showed greater limb activity at 25 than at 35°C.

Furthermore, whereas CLON continued to cause high levels of activity at 16 days, limb activity at this age for the CDZ and DZP treated rats declined to the level observed in VEH treated rats. This confirms our earlier observations of a "paradoxical" activating effect of the benzodiazepines in rats up to 8–12 days of age and shows in addition, that this effect occurred at a low test temperature but not at one which approximated the nest. Our finding that CLON was activating primarily at the lower temperature is the opposite result to that reported earlier by Reinstein and Isaacson [14]. but these investigators tested a considerably higher dose, 2 mg/kg.

Both CLON and the benzodiazepines at the doses which were found here to activate infant rats, suppress activity in adult rats. The sedative effect of CLON in adults (Drew *et al.* [3]) as well as its locomotor activating effects in infant rats are blocked by alpha two adrenoceptor antagonists [9 14]. It would be instructive to determine if the "paradoxical" activating effects of the benzodiazepines in infant rats also requires normal functioning of these receptors. Both CLON [2] and the benzodiazepines (Grant *et al.* [5]) inhibit the firing of the locus coeruleus in the adult rat. This inhibitory effect of CLON is mediated through alpha two adrenoceptors while that of the benzodiazepines however may occur possibly via GABA receptors [4]. It could be the case that the reversal of the effects of CLON and the benzodiazepines from behavioral activation to inhibition as the rat matures, reflects a change in the consequences of locus coeruleus inhibition with maturation.

Do benzodiazepines cause "behavioral convulsions" in infant rats? Clearly our data confirm our earlier observations (Pusztay *et al.* [11]) and those of Barr and Lithgow [1] showing that they elicit an intense "paradoxical" locomotor activation in very young rats. PTZ is a prototypical and reasonably well characterized chemical convulsant [16] and it would appear from our data that if the benzodiazepines induce seizures in young rats, then at the very least the behavioral expression of these seizures is clearly distinct from those elicited by PTZ. Thus we found that whereas PTZ was most activating at the higher test temperature, the opposite was true for the benzodiazepines. Furthermore while PTZ elevated scores on a behavioral composite selected A

PRIORI to reflect seizures, the benzodiazepines did not elevate these scores. Additional differences included the inhibition of the righting reflex by the benzodiazepines but not by PTZ and the evocation of vocalizations after PTZ but not by the benzodiazepines. Finally, research in this laboratory shows that the benzodiazepines effectively block PTZ induced seizures in very young rats (Pusztay and Pappas, unpublished data). On the basis of behavioral similarity, CLON's effect was more like that of PTZ. It was the only other drug to increase seizures scores and vocalizations. It does like the benzodiazepines however, block PTZ induced seizures in rats as young as 15 days (Smythe and Pappas, unpublished observations) when it still causes intense behavioral activation. We suggest therefore, that if the benzodiazepines induce brain seizures in young rats, that these seizures are behaviorally distinct from and antagonistic to those caused by PTZ. It would be premature to conclude that they do not induce brain seizures in young rats since the latter can be clearly discerned only through examination for paroxysmal brain activity after their administration.

ACKNOWLEDGEMENTS

This research was supported by grants from the Ontario Mental Health Foundation and from NSERC to the first author. We thank Boehringer Ingelheim (Can.) for their donation of clonidine and Hoffman La Roche (Can.) for their donation of chlordiazepoxide and diazepam. We thank Ray Ings, Bob Bialik, Cathy Ryan and Jim Smythe for their assistance and advice.

REFERENCES

1. Barr, G. A. and T. Lithgow. Effect of age on benzodiazepine-induced behavior convulsions in rats. *Nature* **302:** 431–432, 1983.
2. Cedarbaum, J. M. and G. K. Aghajanian. Catecholamine receptors on locus coeruleus neurons: pharmacological considerations. *Eur J Pharmacol* **44:** 375–385, 1977.
3. Drew, G. M., A. J. Gower and A. S. Marriot. Alpha two adrenoceptors mediate clonidine-induced sedation in the rat. *Br J Pharmacol* **67:** 133–141, 1979.
4. Grant, S. J. and D. E. Redmond, Jr. The neuroanatomy and pharmacology of the nucleus locus coeruleus. In: *Psychopharmacology of Clonidine*, edited by H. Lal and S. Fielding. New York: Alan R. Liss, 1981, pp. 1–27.
5. Grant, S. J., Y. H. Huang and D. E. Redmond. Benzodiazepines attenuate single unit activity in the locus coeruleus. *Life Sci* **27:** 2231–2236, 1980.
6. Horwitz, J., A. Heller and P. C. Hoffman. The effect of development of thermoregulatory function on the biochemical assessment of the ontogeny of neonatal dopaminergic neuronal activity. *Brain Res* **235:** 245–252, 1982.
7. Millichap, J. G. Seizure patterns in young animals. Significance of brain carbonic anhydrase II. *Proc Soc Exp Biol Med* **97:** 606–611, 1958.
8. Moorcroft, W. H., L. D. Lytle and B. A. Campbell. Ontogeny of starvation-induced behavioral arousal in the rat. *J Comp Physiol Psychol* **75:** 59–67, 1971.
9. Nomura, Y. and T. Segawa. The effect of α-adrenoceptor antagonists and metiamide on clonidine-induced locomotor stimulation in the infant rat. *Br J Pharmacol* **66:** 531–535, 1979.
10. Pappas, B. A., J. V. Gallivan, T. Dugas, M. Saari and R. Ings. Intraventricular 6-hydroxydopamine in the newborn rat and locomotor responses to drugs in infancy: no support for the dopamine depletion model of minimal brain dysfuntion. *Psychopharmacology (Berlin)* **70:** 41–46, 1980.
11. Pusztay, W. J., B. A. Pappas and R. Ings. Diazepam elicits hyperactivity in infant rats. Presented at Annual Meeting of the International Society for Developmental Psychobiology, St. Louis, 1979.
12. Reinstein, D. K. and R. L. Isaacson. Clonidine sensitivity in the developing rat. *Brain Res* **135:** 378–382, 1977.
13. Reinstein, D. K. and R. L. Isaacson. Behavioral components of the clonidine response in the developing rat. *Soc Neurosci Abstr* **5:** 175, 1979.
14. Reinstein, D. K. and R. L. Isaacson. Behavioral and temperature changes induced by clonidine in the developing art. *Neurosci Lett* **26:** 251–257, 1981.
15. Vernadakis, A. and D. M. Woodbury. The developing animal as a model. *Epilepsia* **10:** 163–177, 1969.
16. Woodbury, D. M. Convulsant drugs: mechanisms of action. In: *Antiepileptic Drugs: Mechanisms of Action*, edited by G. H. Glaser, J. K. Penry and D. M. Woodbury. New York: Raven Press, 1980, pp. 249–303.