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Increased Sensitivity to Depolarizing and Nondepolarizing Neuromuscular Blocking Agents in Young Rat Hemidiaphragms

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Background: **Newborn neuromuscular junctions are more sensitive to *d*-tubocurarine than more mature preparations. It is unclear whether the same modifications occur with newer nondepolarizing agents and depolarizing agent succinylcholine. The purpose of this study was to determine the relative sensitivity of newborn neuromuscular junctions to succinylcholine and five nondepolarizing agents.**

Methods: **The phrenic nerve–hemidiaphragm preparation from 60 rats was used, 30 aged 9–12 days (newborn) and 30 aged 27–33 days (adult). Five rats from each group were exposed to one of six neuromuscular blocking agents (*d*-tubocurarine, cisatracurium, atracurium, vecuronium, rocuronium, and succinylcholine). Indirectly elicited twitch tension was measured during control conditions in the absence of blocking agent, followed by four concentrations of one of the six agents. Concentration–response curves were constructed and the EC_{50} (concentration required to produce 50% depression of twitch tension) was obtained. Potency ratios ($EC_{50adult}/EC_{50newborn}$) were derived for each agent.**

Results: **Newborn preparations were significantly ($P < 0.001$) more sensitive than their adult counterparts for all six agents tested. For nondepolarizing agents, the potency ratio was in the 6–12 range. The $EC_{50adult}/EC_{50newborn}$ were as follows, in decreasing potency order: *d*-tubocurarine, 1.68/0.23 μM; cisatracurium, 2.73/0.47 μM; vecuronium, 5.47/0.59 μM; rocuronium, 9.7/0.78 μM; and atracurium, 12.3/1.9 μM. Succinylcholine was three times as potent in newborn rats, with an $EC_{50adult}/EC_{50newborn}$ of 21.3/7.3 μM. The ratio for succinylcholine was significantly less than for all nondepolarizing drugs ($P < 0.02$).**

Conclusion: **The newborn neuromuscular junction of the rat shows an increased sensitivity to all neuromuscular blocking agents tested, including succinylcholine. However, the potency ratio was greater for nondepolarizing than depolarizing drugs. The optimal dose of these agents for certain situations such as cesarean section and anesthesia in neonates should be reassessed.**

DOSE–response studies indicate that infants require less nondepolarizing neuromuscular blocking drugs, on a milligram per kilogram basis, than older children do. This sensitivity appears to be greatest in neonates (< 1 month old).[1] For succinylcholine, the required dose in neonates and infants seems to be larger than for older children.[2] In addition, the newborn neuromuscular junction has different characteristics compared with that of an older child or an adult. Premature infants and newborns, for example, show fade to tetanic stimulation in the absence of neuromuscular blocking agents, suggesting a reduced safety factor.[3] To determine the sensitivity of neuromuscular junctions to different blocking agents, the relation between concentration of drug and level of blockade must be measured.

For nondepolarizing neuromuscular blocking drugs, pharmacokinetic differences are also present in infants. However, there are few investigations dealing with the very young. Two studies in 0- to 30-day-old humans have been performed, and in both cases, *d*-tubocurarine was used.[4,5] Both studies reported an increased distribution volume at steady state and longer elimination half-life in the neonate compared with the adult. However, there were discrepancies with respect to the sensitivity of the neuromuscular junction, as expressed by the concentration at steady state necessary to produce a 50% depression of the first twitch in the train-of-four (Cp_{ss50}). In one of these reports,[4] a decreased Cp_{ss50} was found, *i.e.*, the neonate was more sensitive. In the other study,[5] neonates were distributed into two groups, one group being more sensitive than adults and the other as sensitive as adults. At present, there are no data on the pharmacokinetics and pharmacodynamics of newer nondepolarizing agents or on succinylcholine in human neonates.

These studies are difficult to perform because few healthy subjects undergo anesthesia and surgery in that age group, the amount of blood that can be withdrawn safely is small, and results might be blurred by maturation processes that occur during the first month of life. In this respect, animal preparations might be useful to gain insight into the maturation process. Meakin *et al.*[6] examined the effect of *d*-tubocurarine on developing diaphragm neuromuscular junctions of rats aged 0–46 days. Their results show that, after birth, the EC_{50} of *d*-tubocurarine, or concentration corresponding to a 50% decrease in twitch height, decreased after birth until a minimum is reached at 5–12 days. At this point, the EC_{50} begins to increase sixfold to eightfold and reaches a plateau at 30 days.

It is unclear whether these results apply to nondepolarizing neuromuscular blocking agents other than *d*-tubocurarine, and what the response to succinylcholine is in this preparation. Therefore, the study was designed to examine the sensitivity of the rat neuromuscular junc-

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tion to five nondepolarizing agents (*d*-tubocurarine, cisatracurium, atracurium, vecuronium, rocuronium) and to one depolarizing agent (succinylcholine), comparing responses in the newborn (9–12 days) and adult (27–33 days) junctions.

Materials and Methods

Animal Preparations

Experiments were conducted on 60 rat left phrenic nerve–hemidiaphragm preparations. The protocol was approved by the Animal Ethics Committee of the Université de Montréal according to CPA rules. Sprague-Dawley rats were killed by decapitation according to the standards of the animal care committee of our institution. Two groups were studied: a newborn group aged 9–12 days and an adult group aged 27–33 days. Each group (n = 30) was divided into six subgroups of five preparations, each exposed to only one neuromuscular blocking agent (*d*-tubocurarine, cisatracurium, atracurium, vecuronium, rocuronium, or succinylcholine). The thorax and upper abdomen were excised *en bloc* and immersed into oxygenated (95%O_2, 5% CO_2) modified Ringer's solution (113 mM NaCl, 4.7 mM KCl, 1.4 mM $CaCl_2$, 0.9 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, and 11. 5 mM glucose) at controlled temperature (36.4–37.2°C) and pH (7.35–7.42). The left phrenic nerve and corresponding hemidiaphragm were isolated under microscope for further dissection. The phrenic nerve was separated from the point of branching near the hemidiaphragm surface. Care was taken to avoid any stretching of the nervous tissue during that phase. Next, the left hemidiaphragm was carefully freed from the connective tissue loosely covering the thoracic side of the muscle, avoiding any traumatic puncture of the muscle fibers. Once phrenic nerve and hemidiaphragm were separated of their connective sheaths, the heart and corresponding vertebral bodies were taken out. Excess thoracic and abdominal wall bones and muscles were cut down to leave only a 3- to 4-mm-high costal wall. Finally, ribs were detached from the remaining vertebral bodies, and the left hemidiaphragm was separated from the rest of the diaphragm, preserving the middle diaphragmatic ligament from vertebral bodies to the xyphoid process of the sternum. Left phrenic nerve–hemidiaphragm preparations were then mounted in a specially designed 5-ml laminar flow chamber filled with oxygenated modified Krebs solution. Preparations were attached to a force displacement transducer by a platinum wire, stretched from a Sylgard support allowing for optimal flow over both thoracic and abdominal faces of the hemidiaphragm, and left to stabilize for 30 min in the bath before beginning experimentation. During that period, preparations were bathed with oxygenated modified Krebs solution kept at 37°C through a thermocouple apparatus.

The phrenic nerve was suctioned into a stimulating electrode and stimulated with supramaximal square wave impulses of 0.2-ms duration using a Grass S88 stimulator (Grass Instruments, Quincy, MA). Train-of-four stimulation (2 Hz for 2 s) was applied every 12 s. The preparation was stretched until maximum output tension was recorded after stimulation. An additional 30 min of stabilization elapsed before the experiment was started. Evoked twitches were measured by a force transducer connected to a low-noise bridge amplifier, displayed on a digital storage oscilloscope, digitized, and recorded on tape. Computer analysis of raw data was performed with AXON (Axon Instruments, Foster City, CA). The first twitch in a train was measured as a percentage of the initial twitch height produced during agent-free perfusion.

Experimental Protocol

In preliminary experiments, a few adult and newborn preparations were exposed to a large number of predetermined concentrations, to target the approximate concentrations producing 20, 40, 60, and 80% block. Neuromuscular blocking agents were kept refrigerated, and final dilutions were not prepared until immediately before each new concentration step. Each experiment was conducted by exposing the preparation to four increasing concentrations expected to yield 20, 40, 60, and 80% of twitch depression in a stepwise fashion, allowing 30 min of equilibration between each step increase. Measurements of twitch tension were made at the end of each equilibration period and after each experiment in drug-free perfusate. Data were analyzed only if mechanical twitch tension returned to 90–110% of baseline recording.

First twitch height (T1) at each concentration step was expressed as percentage of control baseline. For each experiment, a regression curve was generated by plotting twitch depression *versus* the logarithm of the drug concentration in the bathing solution, and the EC_{50} was determined. The mean of the five EC_{50} values for adult and newborn preparations were compared for each drug using the Student *t* test. In addition, mean values of T1 for each concentration in five adult and five newborn preparations were calculated and plotted on a semilogarithmic graph with its SE of T1 depression.

Statistics

For each drug, the ratio of EC_{50} for adult preparations to newborn preparations ($EC_{50adult}/EC_{50newborn}$) was calculated. To compare ratios, the logarithm of each EC_{50} value was obtained, and a mean (± SEM) difference between logarithms (equivalent to ratios) was obtained. These values for all six neuromuscular blocking agents were compared using one-way analysis of variance, and comparisons between groups was performed using the Tukey test, if the groups were statistically different by

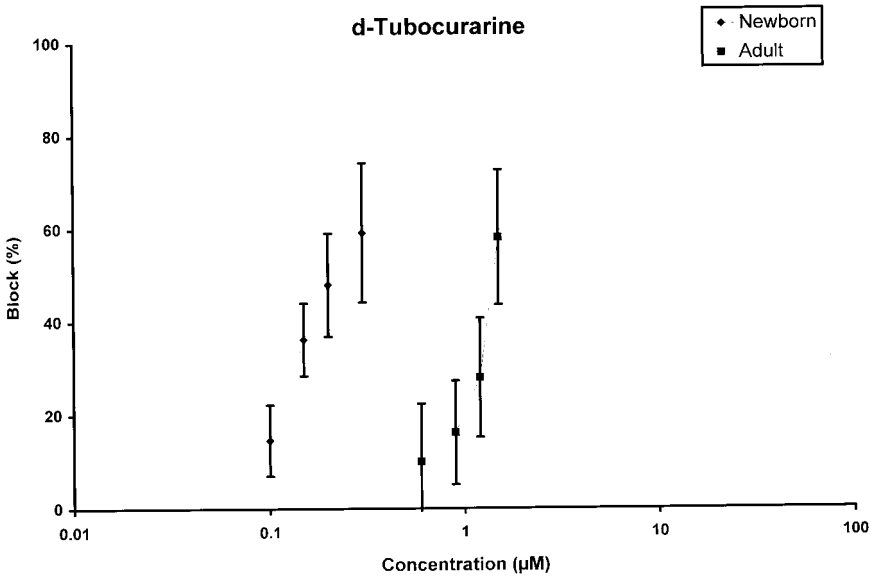

Fig. 1. Neuromuscular block, expressed as percentage of first twitch depression in the train-of-four as a function of *d*-tubocurarine concentration, in the rat phrenic nerve–hemidiaphragm preparation in 9- to 12-day-old animals (newborn) and 27- to 33-day-old animals (adult). For each concentration, mean ± SD block is presented.

analysis of variance. $P < 0.05$ was considered to indicate statistically significant differences.

Results

No train-of-four fade was observed in either newborn or adult preparations before application of neuromuscular blocking agents. For all drugs tested, the EC_{50} was smaller for newborn preparations. The *d*-tubocurarine curve for the newborn group was shifted to the left of the adult group curve, indicating lower concentrations of the blocking agent needed to produce equivalent block in young animals (fig. 1). The EC_{50} for *d*-tubocurarine was 7.3 ± 1.5 times lower in the newborn group (table 1). Cisatracurium was less potent than *d*-tubocurarine in the rat. Its dose–response curve for newborn preparations was also shifted to the left (fig. 2), the EC_{50} for newborn preparations being 5.8 ± 0.5-fold lower than in older rats (table 1). Atracurium had one fourth to one fifth the potency of cisatracurium. The dose–response curve for newborn preparations was shifted to the left (fig. 3), and the EC_{50} was 6.5 ± 0.7 times lower in young than older rats (table 1).

The steroidal agents vecuronium and rocuronium also presented a shift of the newborn dose–response curve to the left (figs. 4 and 5). Vecuronium had approximately twice the potency of rocuronium in rats. The EC_{50} values for newborn preparations were 9.3 ± 1.6 times lower for vecuronium and 12.5 ± 1.4 times for rocuronium than in adult animals (table 1).

Figure 6 shows that, for the depolarizing agent succinylcholine, the dose–response curve was also shifted to the left, representing higher sensitivity of newborn preparations to this agent. The EC_{50} for succinylcholine was the highest of all drugs tested, 7.3 and 21.3 μM, respectively, for adult and newborn preparations (table 1). The ratio of the EC_{50} was 2.9 ± 0.4, lower than all nondepolarizing agents ($P < 0.02$).

Table 1. Effective Concentrations (EC_{50}) for 50% Blockade for Various Neuromuscular Blocking Drugs in Adult and Newborn Preparations, with $EC_{50adult}/EC_{50newborn}$ Ratios

Relaxant	EC_{50} (μM) (Mean ± SD)		Ratio (Mean ± SEM)
	Adult	Newborn	
Depolarizing			
1. Succinylcholine	21.3 ± 1.3	7.3 ± 2.3	2.9 ± 0.4[2,3,4,5,6]
Nondepolarizing			
2. *d*-Tubocurarine	1.68 ± 0.52	0.23 ± 0.08	7.3 ± 1.5[1]
3. Atracurium	12.3 ± 2.5	1.90 ± 0.26	6.5 ± 0.7[1,6]
4. *cis*-Atracurium	2.73 ± 0.23	0.47 ± 0.09	5.8 ± 0.5[1,6]
5. Vecuronium	5.47 ± 0.45	0.59 ± 0.22	9.3 ± 1.6[1]
6. Rocuronium	9.7 ± 0.7	0.78 ± 0.20	12.5 ± 1.4[1,3,4]

Number of subjects is five in each group. A superscript indicates $P < 0.05$ (Tukey test) *versus* the agent with the corresponding number.

Discussion

This study shows that, in the rat hemidiaphragm, newborn preparations are significantly more sensitive then their adult counterparts to all neuromuscular blocking agents. The experimental model was designed to compare different concentrations, not dosages, for different agents during controlled conditions. Therefore, the pharmacodynamic properties, *i.e.*, the relation between concentration and effect, of the drugs were studied. Although the potency ratio for succinylcholine was the smallest of all the compounds tested, newborn rats were still three time as sensitive to succinylcholine as older animals. For all nondepolarizing agents, the difference between adult and newborn preparations was even greater (potency ratio between 6 and 12). Absence of fade in newborn hemidiaphragms and increased re-

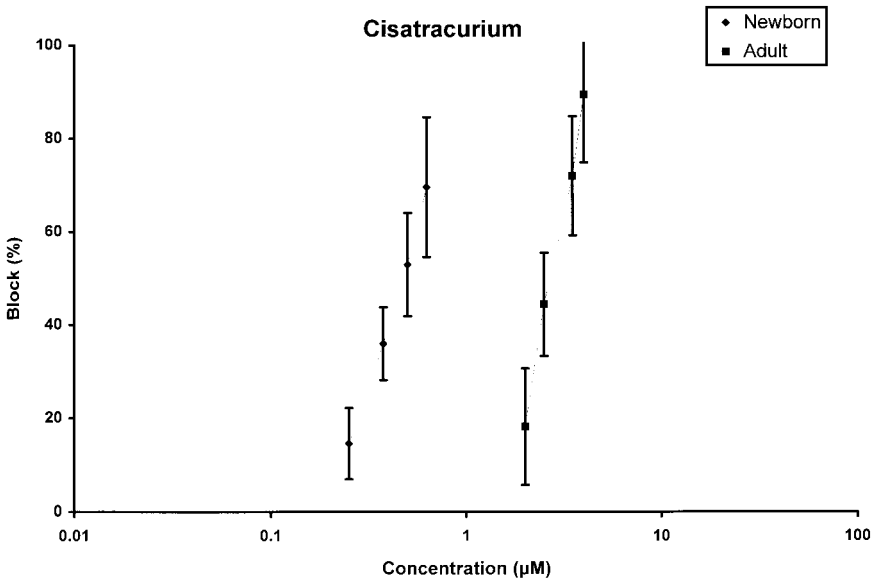

Fig. 2. Neuromuscular block, expressed as percentage of first twitch depression in the train-of-four as a function of cisatracurium concentration, in the rat phrenic nerve–hemidiaphragm preparation in 9- to 12-day-old animals (newborn) and 27- to 33-day-old animals (adult). For each concentration, mean ± SD block is presented.

sponse to succinylcholine compared with adult preparations suggest that the increased sensitivity is not a myasthenic-like process. Because the number of subjects ineach group was too small to compare parallelism of the dose–response curves, it is difficult to determine whether two populations of receptors were present in the newborn.

In a previous study, Meakin *et al.*[6] measured the effect of *d*-tubocurarine on maturing neuromuscular junctions by exposing phrenic nerve–hemidiaphragm preparations from rats ranging in age from a few hours to 46 days to determine the EC_{50} of each subject. The relation between EC_{50} and age shows a minimum between 5 and 12 days of life, when the neuromuscular junction in the rat is most sensitive to neuromuscular blocking agents. After 25 days of life, the *d*-tubocurarine EC_{50} plateaus at values approximately five times higher than the minimum observed at 5–12 days. Meakin *et al.*[6] concluded that the phrenic nerve–hemidiaphragm preparation of 11-day-old rats constitutes an interesting model for the study of the effects of neuromuscular blocking agents on neonatal structures. These results prompted us to use 9- to 12-day-old preparations as the most sensitive stage of development for the neuromuscular junction in rats, and to compare them with 27- to 33-day-old preparations, as they seemed to represent the onset of maturity. The EC_{50} values obtained by Meakin *et al.*[6] for *d*-tubocurarine on adult and newborn preparations (0.8 and 0.16 μM, respectively) are comparable to ours (1.68 and 0.23 μM, respectively), and the ratios are almost identical.

The rat phrenic nerve–hemidiaphragm preparation was used in this study chiefly because its response during the maturation process was well characterized.[6] It is a mammalian preparation that has similarities with hu-

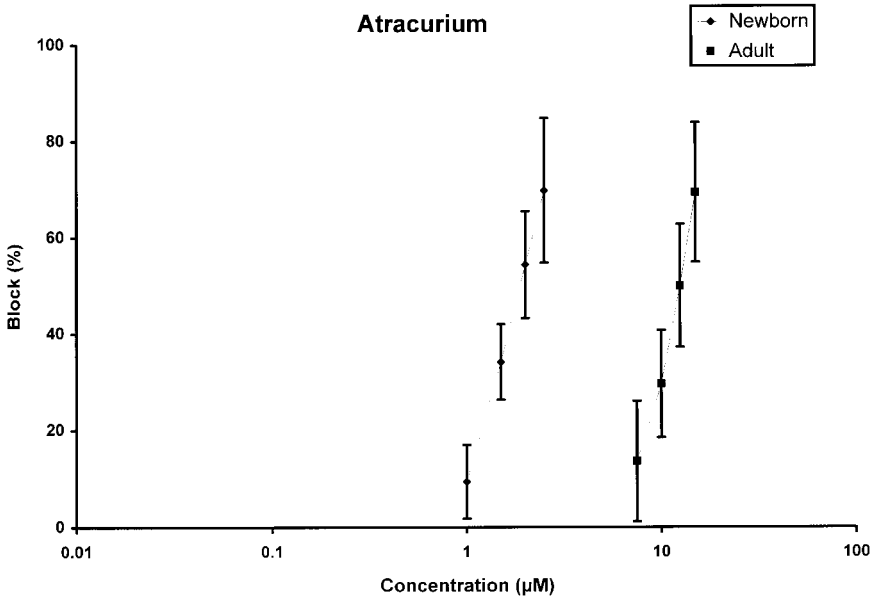

Fig. 3. Neuromuscular block, expressed as percentage of first twitch depression in the train-of-four as a function of atracurium concentration, in the rat phrenic nerve–hemidiaphragm preparation in 9- to 12-day-old animals (newborn) and 27- to 33-day-old animals (adult). For each concentration, mean ± SD block is presented.

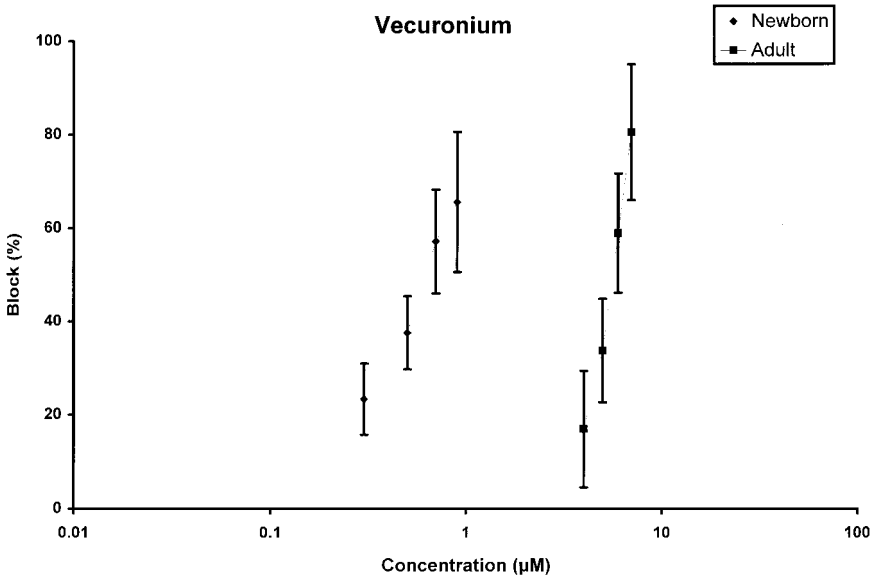

Fig. 4. Neuromuscular block, expressed as percentage of first twitch depression in the train-of-four as a function of vecuronium concentration, in the rat phrenic nerve–hemidiaphragm preparation in 9- to 12-day-old animals (newborn) and 27- to 33-day-old animals (adult). For each concentration, mean ± SD block is presented.

man neuromuscular junctions. In addition, it involves the diaphragm, which is the most important muscle of respiration. These advantages make this model appropriate in neuromuscular studies. Of particular value is the ability to control the environment in which the experiments are conducted.

The experimental setup allowed us to control physiologic conditions precisely. Chemical composition of the bathing is critical, as shown by Foldes.[7] All of our experiments were performed in Krebs solution. Because hypothermia potentiates the effect of steroidal agents without affecting the potency of benzylisoquinolinium agents,[8] temperature was measured and maintained within a narrow range (36.4–37.2°C). Hypoxia can produce failure of neuromuscular transmission in the developing diaphragm and dysfunction in more mature preparations.[9] For this reason, oxygenation of the bathing solution was optimized. Two of the neuromuscular blocking agents used (atracurium and cisatracurium) undergo Hofmann degradation at a rate that depends on temperature and pH. This is why a fresh solution was prepared immediately before each concentration change, and the perfusate was kept at 18°C until it entered the perfusion chamber to minimize spontaneous degradation. Furthermore, because the same set-up was used for both the newborn and adult preparations, the degree of degradation was likely to be the same in each case, preserving the ratio between the two preparations. Our pilot studies clearly demonstrated that variation in calcium and magnesium concentrations changed the amplitude of recorded signals, lower temperature decreased signal amplitude, and uneven flow over both surfaces of the muscle produced regional neuromuscular dysfunction evidenced by localized dyskinesia or even akinesia. Care was taken to use the same ionic concentration in all solutions and to obtain an even flow on both

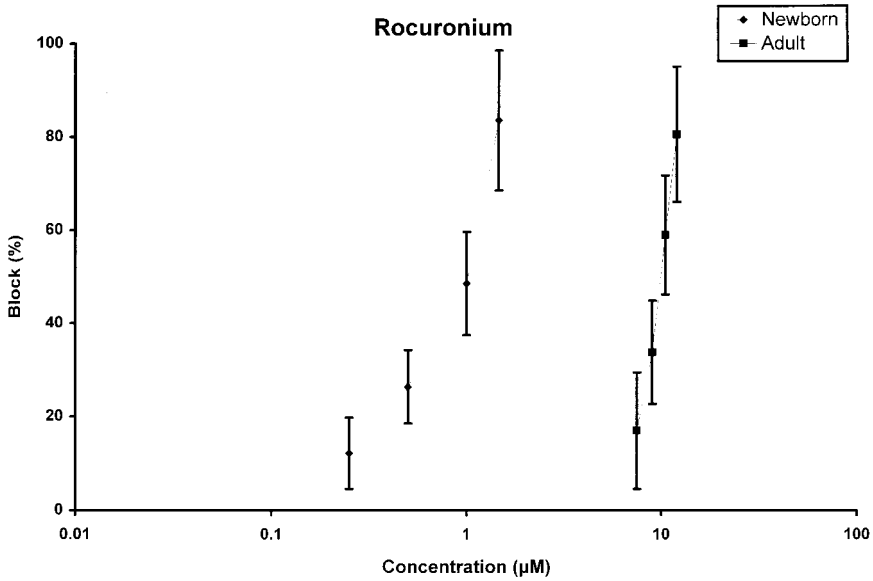

Fig. 5. Neuromuscular block, expressed as percentage of first twitch depression in the train-of-four as a function of rocuronium concentration, in the rat phrenic nerve–hemidiaphragm preparation in 9- to 12-day-old animals (newborn) and 27- to 33-day-old animals (adult). For each concentration, mean ± SD block is presented.

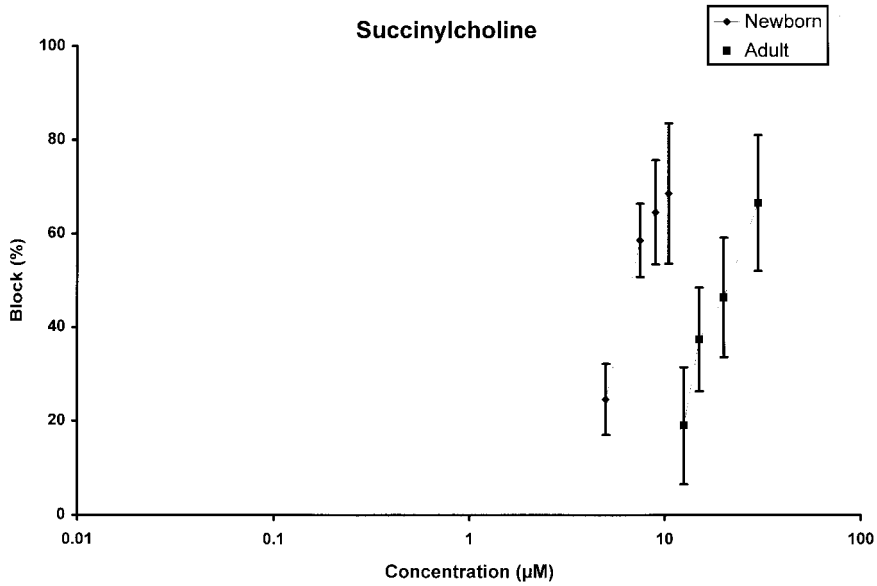

Fig. 6. Neuromuscular block, expressed as percentage of first twitch depression in the train-of-four as a function of succinylcholine concentration, in the rat phrenic nerve–hemidiaphragm preparation in 9- to 12-day-old animals (newborn) and 27- to 33-day-old animals (adult). For each concentration, mean ± SD block is presented.

sides of the muscle. Finally, data were retained for statistical analysis only when twitch height returned to within 10% of the initial value at the end of each experiment.

At birth, several neuromuscular junctions can be found on each muscle fiber, and fetal-type receptor, which has a γ subunit, predominates over the adult type, which contains an ε subunit.[10,11] The period of increased sensitivity to neuromuscular blocking drugs (9–12 days) coincides with the completion of the switch from polyinnnervation to monoinnervation and by replacement of fetal receptors by adult receptors.[10,11] Thus, the increased sensitivity is unlikely to be caused by different gating and electrical properties of fetal (γ)-type receptors. It seems more likely that structural changes of the presynaptic apparatus,[12] density of postsynaptic components,[11,13] or functional safety factors[14] might play a role. For example, development of the neuromuscular junction in the rat is first characterized by the atrophy of all but one synapse. Then the density of receptors at the remaining endplate increases markedly, as it decreases in extrajunctional areas, synaptic folds enlarge and develop, and the size of the neuromuscular junction increases.[11] The relation between sensitivity to vecuronium in different muscles of the cat was found to correlate with the number of receptors.[15] The same principle might apply to the rat hemidiaphragm, where increased sensitivity to nondepolarizing neuromuscular blockers also occurs when the number and density of receptors at the endplate are relatively small.

Because the mechanism of action of succinylcholine is uncertain, interpretation of the results is difficult. The newborn preparations were expected to be less sensitive to the drug because it is an agonist that presumably would be less effective if the number of receptors are reduced. However, succinylcholine probably has other mechanisms of action, such as inactivation of sodium channels.[16] Presynaptic effects might also be important.[16]

The development of the human neuromuscular junction is qualitatively similar to that in the rat, but the time course is different. Polyinnervation, for example, could be a confounding factor during our observations. Nevertheless, as previously discussed by Sanes and Lichtman,[11] synapse elimination is dependent on strong synaptic input; this is certainly the case for the diaphragm, which after birth shows 5–15% elimination per day in the rat. Thus, the contribution of polyinnervation in 9- to 12-day-old rats is probably physiologically insignificant. Hesselmans *et al.*[17] demonstrated that the fetal γ subunit could not be found later than 31 weeks of gestation in humans, indicating that transition to the ε or adult type is normally complete well before birth. However, neuromuscular junction geometry (size of endplates and folds) does not achieve adult configuration until the twelfth week after birth,[3] or perhaps 1 year of life.[17] The exact time corresponding to maximum sensitivity of the neuromuscular junction in humans is not known with certainty, but currently available evidence suggests that it is probably some time between 31 weeks gestation and 12 weeks of extra uterine life. Even if rat neuromuscular junctions have been generally accepted as a suitable model for further investigation of the action of neuromuscular blocking agents during maturation, extension of the results between species must be made with caution.

For succinylcholine, the EC_{50} in young rats was less then in adults. However, the ED_{50} and recommended doses for neonates and infants are greater than for older children.[2] This apparent discrepancy cannot be explained by the pharmacodynamic properties determined in this study. The explanation must reside, at least in part, in a greater volume of distribution for this agent in

the human neonate, a pharmacokinetic property. The volume of distribution in the neonate has never been measured for succinylcholine, but it is reasonable to assume that it is approximately equal to the extracellular volume, which is greater in the neonate than in the adult.

For nondepolarizing agents, the ED_{50} in neonates is less than, or approximately the same as, in older children or in adults.[18] This appears to contradict the results of the current study, where concentrations for blockade were markedly lower in young rats. However, the extracellular fluid volume and volume of distribution of neuromuscular blocking drugs, expressed in milliliters per kilogram, are greater in neonates than in older individuals.[4] This could suggest that the concentration required for blockade is less in neonates than in more mature subjects. This is supported by pharmacokinetic studies performed in neonates, where concentrations of *d*-tubocurarine required for 50% blockade were less in neonates than in older children.[4] We did not observe a lower sensitivity to the nondepolarizing agent *d*-tubocurarine in our study, as reported by Matteo *et al.*[5] These discrepancies might be a result of interpatient variability and the maturation process, which probably occurs between the age of 0 and 100 days. Finally, our data indicate that there seems to be homogeneity between all nondepolarizing agents in their blocking potency in newborn subjects. In other words, no individual nondepolarizing drug seems to spare the neonate compared with the adult.

The results of this study might have special relevance for the use of neuromuscular blocking agents in the pediatric population. First, it illustrates that, at a critical time during development, the neuromuscular junction might be very sensitive to blocking agents. Second, the doses of nondepolarizing agent required for newborns are only slightly lower than for older children, not because their neuromuscular junction has the same sensitivity, but because the newborn distribution volume is larger. During a cesarean section, the newborn is not given a dose, but is exposed to its mother's blood concentration. The fetal concentration depends on the dose given to the mother, the umbilical/maternal ratio, and the induction–delivery interval.[19,20] Residual paralysis might be difficult to detect because loss of tone in a baby can have other causes than neuromuscular relaxants. Perreault *et al.*[21] observed a lower neurologic and adaptive capacity score in newborns of women who received atracurium than in those who received succinylcholine. Succinylcholine is by far the most widely used agent that presents the most favorable potency ratio, and this might explain, in part, the absence of any apparent clinical problem. This situation could change if the use of succinylcholine decreases and the drug is replaced by nondepolarizing agents. Premature infants might be at particular risk, if, as suggested by Berde and Cairns,[22] the first 7 days of postnatal life in rats correspond to the last 16 weeks of gestation in humans as far as the development of the peripheral nervous system is concerned. Our results clearly demonstrate different pharmacodynamic properties for the newborn neuromuscular junction with regard to potency ratio of blocking agents. There is no evidence of pharmacodynamic resistance to succinylcholine in the newborn, leaving only pharmacokinetic properties such as distribution volume to explain the clinical response observed in the newborn. Much work remains to measure the relative weight and chronologic importance of pharmacologic characteristics of neuromuscular blocking agents during development in the newborn and in the pediatric population in general.

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