blockers of these channels did not block the action of SRT-b (Fig. 3B). Other experiments showed that phosphoinositide hydrolysis could be induced by SRT-b in a calcium-free medium, with and without 1 m*M* EGTA (Fig. 3C). Under the two latter conditions the efficacy of the toxin was lower than that observed in the presence of Ca^{2+}; however, since the basal hydrolysis was also reduced, the ratio of the induced to the basal hydrolysis remained unchanged. Similar effects were observed with the nonproteinaceous maitotoxin, a potent marine toxin found in tropical dinoflagellates (7).

Our results suggest that the sarafotoxins are novel and specific activators of the PDE system. They may exert their activity either by direct activation or via an unidentified class of membrane-bound receptors. While this work was in progress, Yanagisawa *et al.* (*8*) isolated a vasoconstrictor polypeptide from cultured porcine aortic endothelial cells (endothelin), which was proposed as a modulator of voltage-dependent ion channels. The following comparison between the structures of SRT-b and endothelin shows that the two polypeptides have very similar sequences:

SRT-b CSCKDMTDKECLYFCHQDVIW

Endothelin CSCS S L MDKECVYFCHL DI IW

This sequence similarity, together with the similar vasoconstrictor activities of the two polypeptides, suggests that they share a common binding site. It is of course possible (although less likely) that the small differences between endothelin and the sarafotoxins are enough to bring into play different mechanisms of action for their respective vasoconstrictor activities. Whatever their mechanisms of action, it is quite unusual to find such remarkable resemblances in sequence and biological action between an endogenous polypeptide, which exists as a natural component of mammalian endothelium, and one which constitutes a lethal component of snake venom.

Two hypotheses are suggested: (i) that it is the small difference in amino acid sequence between endothelin and sarafotoxins that accounts for the high toxicity of sarafotoxins; and (ii) that endothelin might be as toxic as the sarafotoxins, but its tissue content and activity are well regulated by synthetic and degradative mechanisms. This might imply that the pathology of hypertension and other vasoconstrictor diseases could be associated with an anomalous increase in the concentration of endothelin or related peptides.

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SRT-a CSCKDMSDKECLNFCHQDVIW

SRT-b CSCKDMTDKECLYFCHQDVIW

SRT-c CTCNDMTDEECLNFCHQDVIW

Abbreviations for the amino acid residues are: C, Cys; D, Asp; E, Glu; F, Phe; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; Q, Gln; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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Heterogeneity of Glycine Receptors and Their Messenger RNAs in Rat Brain and Spinal Cord

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Messenger RNAs isolated from adult or newborn rat spinal cord were fractionated in a sucrose gradient. The fractions were injected into *Xenopus* oocytes to determine their potencies for expression of glycine receptors (GlyRs), which were then examined electrophysiologically. The sedimentation profiles disclosed two classes of GlyR mRNAs, one heavy and the other light. The adult spinal cord was rich in heavy GlyR mRNA, whereas the light GlyR mRNA was more abundant in neonatal spinal cord and in adult cerebral cortex. Glycine receptors encoded by heavy and light mRNAs of adult spinal cord showed some electrophysiological differences. Thus there are two types of GlyRs encoded by mRNAs of different sizes, and the expression of these mRNAs is developmentally regulated. A tissue- and age-dependent distribution of heterogeneous GlyR mRNAs may imply diverse roles of the GlyRs in neuronal function in the central nervous system.

GLYCINE AND γ-AMINOBUTYRIC ACID (GABA) are the major inhibitory neurotransmitters in the central nervous system of vertebrates; GABA operates mainly in the brain, whereas glycine acts mainly in the spinal cord (*1*). In the

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Table 1. Peak amplitudes of the currents elicited by amino acids in oocytes injected with fractionated mRNAs from the rat. The fraction number of each source corresponds to that shown in Fig. 1. Each value represents the average peak amplitude of the currents (with SEM, in nanoamperes) in oocytes (numbers in parentheses) obtained from one to four different donors. The glycine/β-alanine ratios given are the averages (with SEM) of the ratio obtained in individual oocytes.

Class	Source, fraction no.	GABA (1 m*M*)	Glycine (1 m*M*)	β-Alanine (1 m*M*)	Glycine/ β-alanine ratio
H	Adult cord, Fr. 6	<2.0 (8)	585 ± 97 (16)	253 ± 42 (16)	2.7 ± 0.3
H	Neonatal cord, Fr. 3	<2.0 (6)	203 ± 59 (6)	113 ± 33 (6)	2.2 ± 0.2
H	Adult cortex, Fr. 4	<2.0 (3)	55 ± 11 (3)	37 ± 12 (3)	1.7 ± 0.3
L	Adult cord, Fr. 15*	7.3 ± 1.7 (11)	431 ± 56 (19)	34 ± 6 (19)	13.0 ± 0.9
L	Neonatal cord, Fr. 13	16.0 ± 2.7 (6)	2302 ± 205 (6)	147 ± 14 (6)	15.3 ± 1.2
L	Adult cortex, Fr. 14	114 ± 18 (10)	595 ± 122 (10)	44 ± 9 (10)	15.0 ± 1.9

*For concentration of the mRNA, see (*12*).

adult rat, the distribution of polyadenylated [poly(A)$^+$] mRNAs coding for GABA and glycine receptors parallels that of the transmitters, with the brain richer in GABA receptor mRNA and the spinal cord richer in GlyR mRNA (*2, 3*).

Polyadenylated mRNAs, isolated from adult rat cerebral cortex and spinal cord and from neonatal spinal cord, were fractionated by sucrose density gradient centrifugation (*4*) and injected into *Xenopus* oocytes to express functional neurotransmitter receptors and voltage-operated channels (*5*). The oocytes were examined electrophysiologically 4 to 7 days later to measure the amplitude of the membrane currents elicited by glycine (*6*). The sizes of the currents reflect the relative amount of GlyR mRNA present in the corresponding fractions.

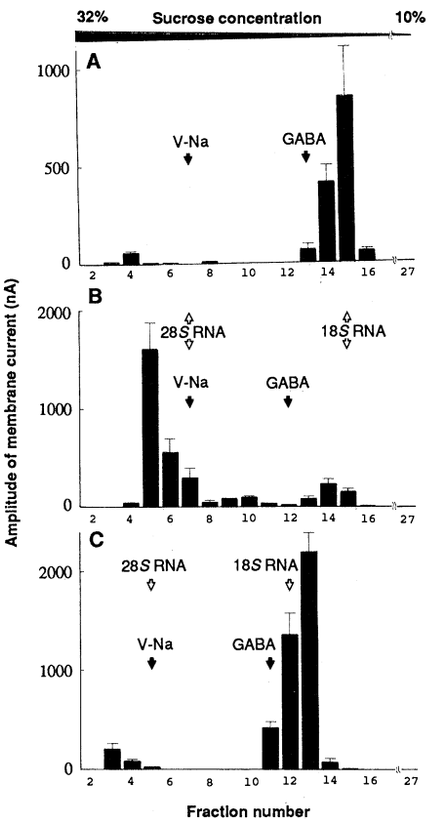

Fig. 1. Expression of glycine receptors by sucrose density gradient fractions of mRNAs from adult rat cerebral cortex (**A**), adult rat spinal cord (**B**), and neonatal rat spinal cord (**C**). The fractionated mRNA was injected into oocytes of *Xenopus laevis* to examine the expressional potency for glycine receptors as well as for other receptors and voltage-operated ion channels. The oocytes were voltage-clamped at −60 mV (*6*). Each column represents the mean peak amplitude of the current induced by 1 m*M* glycine in three to ten oocytes; bars indicate SEM. Positions of mRNAs that induced the largest voltage-dependent Na^+ currents (V-Na) and GABA-induced currents are indicated by filled arrows. The positions of 28*S* and 18*S* rRNA, shown with open arrows, were determined from a parallel gradient loaded with poly(A)$^-$ RNA.

The "expressional potency" of the different fractions of GlyR mRNAs is illustrated in Fig. 1. In agreement with earlier results (*4*), most of the GlyR mRNA in the adult cortex was contained in two fractions sedimenting close to the position of 18*S* ribosomal RNA (rRNA) (~2.0 kb) (Fig. 1A). There was also a smaller peak of heavier GlyR mRNA (Fig. 1A, fraction 4). In contrast, the expressional potency of the adult spinal cord mRNA had the opposite profile (Fig. 1B). Most of the GlyR mRNA sedimented at rates faster than those of the 28*S* rRNA (~5.1 kb) and the mRNA encoding Na^+ channels (~9.0 kb), and a smaller peak was seen close to the 18*S* rRNA [for base sizes of the RNAs, see (*7*)].

One explanation for this difference might be that the spinal cord GlyR mRNA aggregates and thus sediments in the high-density fractions. However, this does not seem to be the case, because, after fractionation in sucrose containing 70% formamide to partly denature the mRNAs, the GlyR mRNA from the spinal cord again sedimented in fractions heavier than the 28*S* rRNA; and the cerebral cortex GlyR mRNA still sedimented close to the 18*S* rRNA (*8*). Thus, in both adult rat spinal cord and brain there seem to be two main classes of GlyR mRNAs, which are differentially distributed. We shall call these heavy (H) and light (L) mRNAs. Although it could be that the H GlyR mRNA corresponds to a heterogeneous nuclear RNA, it seems more likely that it is a fully processed mRNA, in which case its molecular size is considerably greater than the size of the cDNA (~1.4 kb) for a strychnine [a GlyR antagonist (*1*)]-binding protein (*9*). The existence of a large mRNA encoding GlyRs would not be surprising, because some mRNAs coding for K^+ channels, and for an acetylcholine receptor, contain long untranslated portions (*10*). Moreover, the spinal cord of 20-day-old rats contains substantial amounts of large mRNAs (~9, ~6, and ~5 kb) that hybridize with the cDNA encoding the strychnine-binding protein (*9*).

Strychnine-sensitive GlyRs are present in the rat spinal cord even at early postnatal stages (*11*). Moreover, the neonatal (3- to 4-day-old) rat spinal cord is rich in GlyR mRNA, with an expressional potency comparable to that of the adult cord (*3*). Interestingly, however, the profile of the currents elicited by glycine in oocytes injected with fractions of neonatal spinal cord mRNA (Fig. 1C) differed from that of the adult spinal cord and resembled that obtained with cerebral cortex mRNA. This difference suggests that early during development the spinal cord expresses predominantly the L GlyR mRNA and switches later to produce the H mRNA as the major transcript coding for GlyRs.

The properties of the glycine receptors expressed by the H and L mRNAs from the adult spinal cord showed some similarities and also some differences (*12*). For example, in both cases the currents elicited by glycine are carried mainly by Cl^- ions, because they reversed direction when the clamp holding potential was close to −20 mV, which corresponds to the Cl^- equilibrium potential in *Xenopus* oocytes (*13*). Moreover, the current-voltage relation of the responses to glycine showed a marked rectification, with the current decreasing in amplitude at potentials more negative than about −100 mV. These properties are similar to those of human and rat brain GlyRs expressed in oocytes (*2, 14*). The receptors expressed by H and L mRNAs are also similar in their response to pharmacological blockers. For instance, both types of glycine receptors were reversibly blocked by 0.5 μ*M* strychnine (Fig. 2, A and C). Furthermore, picrotoxin, a blocker of Cl^- channels that are gated by GABA receptors (*1*), at a relatively high concentration (20 μ*M*) reduced to approximately the same extent the currents elicited by activation of both types of GlyRs (Fig. 2, B and D). In contrast, bicuculline, a

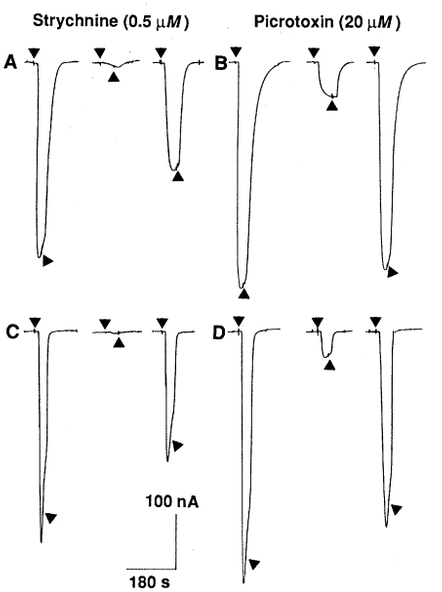

Fig. 2. Blocking actions of strychnine and picrotoxin on the glycine-induced responses. Oocytes were injected with adult spinal cord mRNA fraction 6 (**A** and **B**) or fraction 15 (**C** and **D**) [see (*12*)] and were voltage-clamped at −60 mV. Glycine (1 m*M*) was applied during the periods marked by adjacent pairs of triangles. In each frame the left trace is the control, the middle was obtained in the presence of 0.5 μ*M* strychnine (A and C) or 20 μ*M* picrotoxin (B and D), and the right trace was obtained 10 to 30 min after washing out the drugs. Downward deflections denote inward currents.

potent GABA receptor antagonist (*1*), at concentrations of up to 10 μ*M* had little or no effect on the amplitude of glycine-induced currents (*15*).

Some important differences between the GlyRs expressed by the H and L mRNAs are shown in Fig. 3 and Table 1. For example, activation of the GlyRs coded by the H mRNA gave a steeper dose-response relation [half maximally effective concentration (EC_{50}) of 240 μ*M*] than those coded by the L mRNA (EC_{50} of 680 μ*M*) (Fig. 3A). The initial slopes, obtained from double-logarithmic plots, were 2.45 and 2.43, respectively (Fig. 3A). These values are consistent with those of glycine-induced responses observed in cultured mouse spinal neurons (*16*). Thus it seems that, for both types of GlyRs, more than two glycine molecules need to act simultaneously to open the Cl^- channels.

The GlyRs encoded by the H and L mRNAs also differ in their rates of desensitization. The glycine receptors expressed by spinal cord mRNA, like those expressed by human, rat, or chick brain mRNAs (*2, 3, 14, 17*), mediated currents that were not maintained in the continuous presence of glycine (Fig. 3B). However, the currents elicited by activation of the L GlyR decayed faster than those elicited by activation of the H GlyR (Fig. 3B), and it seems that in both cases the decay followed at least two components.

The agonist β-alanine, an amino acid structurally similar to glycine and GABA, inhibits neuronal activity in the spinal cord (*1*). The oocytes injected with fractions containing H or L mRNA also responded to β-alanine, which generated inward currents. However, they showed markedly different potencies for sensitivity to this agonist (Table 1); namely, the ratio of the glycine- to β-alanine–induced currents (glycine/β-alanine ratio) in the oocytes injected with H mRNA was 2.7 ± 0.3 whereas that in the oocytes injected with L mRNA was 13.0 ± 0.9 (mean ± SEM). It is unlikely that in these cases the β-alanine currents were elicited by activation of GABA receptors, because the β-alanine currents were greatly depressed by 0.5 μ*M* strychnine but not by 10 μ*M* bicuculline. Moreover, the currents generated by GABA were only a few nanoamperes, or were undetectable, in both groups of oocytes (Table 1). A possible explanation for the difference in glycine/β-alanine ratios is that β-alanine binds to the two types of GlyRs with different affinities. However, it is also possible that the spinal cord has an mRNA encoding specific β-alanine receptors, as suggested for rat and chick brain (*17*).

At present, we do not know whether the L GlyR mRNAs of adult cortex and neonatal spinal cord are identical to that of the adult spinal cord; but the receptors expressed by the L mRNA of the three different sources seem similar. For instance, all of the glycine currents were blocked by strychnine (0.5 μ*M*), and the glycine/β-alanine ratios from the three different sources were similar (Table 1).

Both the spinal cord and the brain contain at least two different species of mRNAs that express functionally distinct glycine receptors; but the adult cerebral cortex has predominantly L GlyR mRNA, and the adult spinal cord has mainly H GlyR mRNA. Moreover, expression of the H and L GlyR mRNAs in the spinal cord appears to be developmentally regulated. This is in accord with recent results showing that mRNA derived from the spinal cord of 3-day-old rats hybridizes very little with a cloned cDNA encoding the strychnine-binding subunit of the glycine receptor from the spinal cord of 20-day-old rats (*9*). Several mechanisms could account for the heterogeneity of GlyR mRNAs. For example, the H and L mRNAs may be derived from two different genes, or they may be transcribed from a single gene with alternative processing, including multiple polyadenylation sites as for apolipoprotein B (*18*). Heterogeneity of GlyR mRNAs and their encoded receptors may play an important role not only in neuronal inhibition but also in the development of the central nervous system.

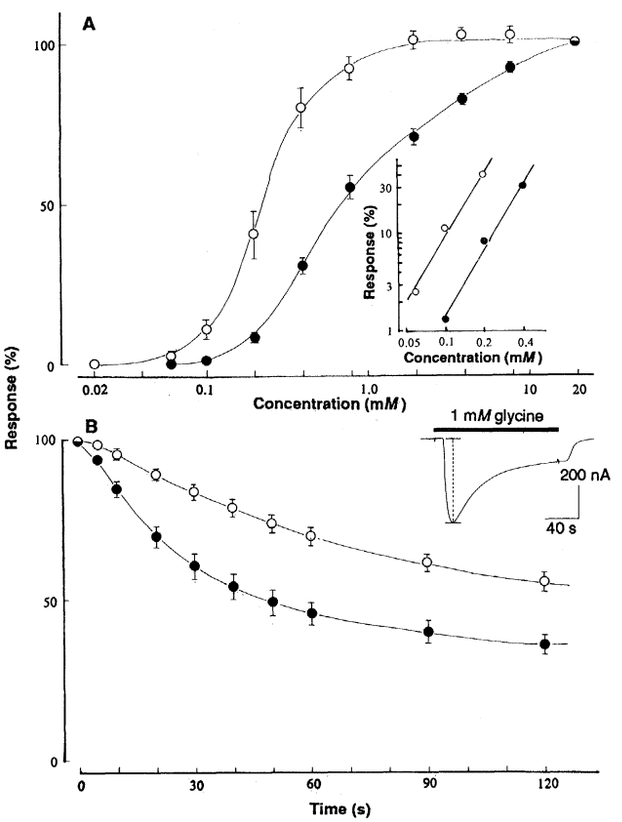

Fig. 3. Dose-response relation (A) and time courses of desensitization (B) of glycine currents in oocytes injected with adult spinal cord mRNA fraction 6 (○) or 15 (●) (*12*). (**A**) The membrane potential was voltage-clamped at −60 mV, and various concentrations of glycine were applied in the bath at 5- to 8-min intervals. The peak amplitude of each response was expressed as a percentage of the response activated by glycine at 20 m*M*. The inset shows double-logarithmic plots at low concentrations of glycine. (**B**) Membrane currents at a clamp potential of −60 mV recorded in the continued presence of glycine (1 m*M*). The size of the inward current was expressed as a percentage of peak amplitude and plotted against time. The inset shows a sample record in an oocyte injected with fraction 15. In (A) and (B), each point represents the mean ± SEM obtained in five to eight oocytes from a single donor. For average amplitudes of the glycine currents shown in this figure, see Table 1.

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6. Preparation of poly(A)$^+$ mRNA from adult (12 to 14 weeks old) rat cerebral cortex and adult and neonatal (3 to 4 days old) spinal cord was as described (*3*). The poly(A)$^+$ RNA (110 to 140 μg) was denatured by heating for 5 min at 65°C, loaded onto a linear sucrose density gradient solution (32 to 10%, w/v, containing 10 m*M* Hepes, 1 m*M* sodium salt of EDTA, and 0.1% lithium laurylsulfate, adjusted to *p*H 7.5) and centrifuged at 260,000*g* for 14 hours at 4°C in a Beckn....1 SW41 rotor. The solution was collected in 0.4-ml fractions, which were precipitated with ethanol and dried. The mRNA fractions were subsequently dissolved in water [volume: 15 μl (adult cortex), 60 μl (adult spinal cord), and 20 μl (neonatal spinal cord)], and 50 nl of each were injected into oocytes. Procedures for electrophysiological experiments were as described (*3, 12*). Two or three sedimentation runs were made for each mRNA source, with similar results.

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Acetaldehyde Production and Transfer by the Perfused Human Placental Cotyledon

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Fetal injury associated with maternal ethanol ingestion is a major cause of congenital anomalies and mental retardation. Studies with animals suggest that acetaldehyde, the primary hepatic oxidative metabolite of ethanol, may contribute to fetal damage. It is not known, however, whether acetaldehyde reaches the human fetus, either by placental production or transfer. Studies utilizing the perfused human placental cotyledon show that the human placenta oxidizes ethanol to acetaldehyde, releasing it into the fetal perfusate. Moreover, when acetaldehyde is present in the maternal perfusate, it is transferred to the fetal side, reaching approximately 50 percent of the maternal level. These findings suggest that the human placenta may play a pivotal role in the pathophysiology of ethanol-associated fetal injury.

ETHANOL INGESTION DURING PREGNANCY may result in fetal injury, including congenital anomalies, intrauterine growth retardation, and mental deficiency (*1*). Ethanol-associated fetal damage is a major cause of mental retardation in the Western world (*1*). However, the pathophysiology of ethanol-associated fetal injury is not well understood. To a large degree, the etiology may involve direct toxicity, with ethanol adversely affecting both fetal development and placental function (*2*). However, acetaldehyde (AcH), the major oxidative product of ethanol, has also been implicated in many of the adverse effects of ethanol consumption (*3*). In pregnant animals treated with ethanol, AcH is found in the fetus (*4*), and administration of AcH to pregnant animals can reproduce the fetal damage seen with ethanol exposure (*5*). Moreover, in humans, the degree of fetal injury may be related to the level of AcH in the maternal circulation (*6*), and there is indirect evidence for maternal-to-fetal transfer of AcH (*7*).

In the experiments described here, we attempted to answer two questions: (i) At clinically relevant ethanol levels, does the human placenta oxidize ethanol so that AcH may appear in the fetal circulation? (ii) At AcH levels attainable in maternal blood, does the human placenta transfer AcH to the fetal circulation? Inasmuch as the human fetal liver has a low capacity for both ethanol and AcH elimination (*8*), the placental delivery of AcH could be a major contributor to the pathogenesis of ethanol-associated fetal injury.

To examine the potential for human placental oxidation of ethanol, we perfused the maternal circulation of a single cotyledon in

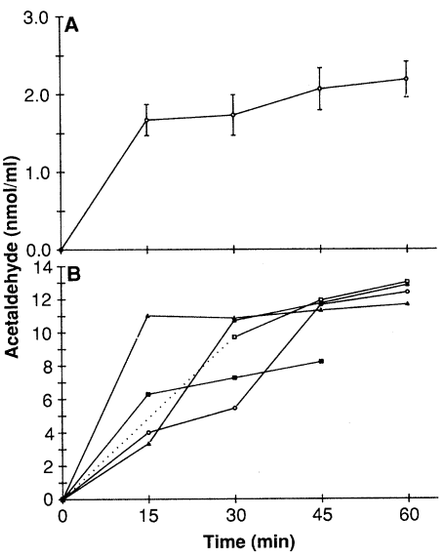

Fig. 1. Acetaldehyde production (**A**) and transfer (**B**) by the perfused cotyledon. Addition of 65 m*M* ethanol to the maternal influx after the equilibration and blank perfusion periods (*14*) resulted in the appearance of AcH on the fetal side (A) ($n = 7$) (mean ± SE). AcH also appeared in the maternal venous efflux (see text). After the perfusion with ethanol alone and a 15-min washout (*14*), both 65 m*M* (300 mg/dl) ethanol and 25 μ*M* AcH were added to the maternal influx and 100 ml of fresh fetal perfusate (recirculated) was analyzed for AcH transfer (B) ($n = 5$) [each transfer pattern is shown; one of the curves did not have a 15-min time point (dotted line)]. The maternal perfusate was nonrecirculated, so that influx concentrations were constant. Perfusate samples (250 μl) were placed in chilled vials containing 250 μl of 1.2*N* perchloric acid and 80 m*M* thiourea, sealed, held on ice for less than 6 hours, and analyzed for AcH and ethanol content by head-space gas chromatography (*9*). Compared to raw perfusate or perfusate with barium hydroxide added, perfusate with perchloric acid–thiourea gave the least spontaneous AcH production. Standards for AcH were prepared from redistilled AcH dissolved in deoxygenated water; we estimated the spontaneous formation of AcH by using fetal-side blank perfusate (*14*), to which varying amounts of ethanol were added. For a given ethanol concentration in the fetal perfusate, a corresponding value for spontaneous AcH formation (determined by linear regression) was subtracted from the total AcH, yielding the net values shown. During maternal perfusion of ethanol alone (A), spontaneous formation accounted for 56.7 ± 3.2% of the total AcH in the fetal perfusate; during perfusion with ethanol and AcH, it accounted for only 11.7 ± 2.0% as a result of the higher AcH levels in the fetal perfusate.

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