COMBINED ACTION OF CARBUTAMIDE AND SODIUM SALICYLATE ON DEVELOPMENT OF RAT EMBRYOS

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There are reports in the literature that both the antidiabetic sulfonamides and salicylates possess a harmful and teratogenic action. The teratogenic activity of carbutamide [15, 16, 21-24] and of sodium salicylate [9, 12, 25] is manifested in rat embryos, and these preparations exhibit their strongest teratogenic effect on the 10th day of pregnancy [1].

Carbutamide and salicylates act similarly on certain metabolic processes in animals. They lower the blood sugar level [3, 5, 6, 17, 19] and possess cytostatic activity [7, 8, 20]. The teratogenic action of salicylates is intensified in stress conditions and with a shift of the acid-base balance toward acidosis [10, 11]. The same effect has been observed in preliminary experiments to study the teratogenic action of carbutamide.

The object of the present investigation was to study the combined action of the antidiabetic sulfonamide carbutamide (N-butyl-N'-sulfanilyurea) and sodium salicylate on the embryogenesis of rats and also to determine whether the teratogenic action of carbutamide is associated with the hypoglycemic activity of the compound.

EXPERIMENTAL METHOD

In the experiments of series I the effect of carbutamide and sodium salicylate on the embryonic development of rats was studied. Experiments were carried out on 159 pregnant rats, of which 12 received the solvent Tween by the gastric route in a dose of 0.5 ml daily from the 1st until the 12th day of pregnancy and 18 acted as controls.

The preparations were given on the 10th day of pregnancy. Carbutamide was given by the gastric route as a suspension in Tween and water, and sodium salicylate was injected subcutaneously as an aqueous solution before and 3 h after administration of the carbutamide. The results were assessed on the 19th day of preganancy, when the number of implantation sites and the number of dead and living (normal and abnormal) embryos were recorded. The numerical results were subjected to statistical analysis.

In the experiments of series II the teratogenic and lethal action of carbutamide in a dose of 3000 mg/kg was studied on the 7th and 10th days of pregnancy in the conditions of artificial hyperglycemia. In these experiments 58 pregnant rats were used. Glucose was given by the gastric route three times in a dose of 6 g per rat: 3 h before, together with, and 3 h after administration of carbutamide.

In the experiments of series III the combined hypoglycemic action of carbutamide and sodium salicylate was studied. Experiments were carried out on 34 male albino rats weighing 120-140 g. Carbutamide was given in doses of 800 and 3000 mg/kg and salicylate in doses of 300 and 600 mg/kg body weight. The blood sugar level was determined by Nelson's method [18] before and 3, 6, and 9 h after administration of the preparations.

EXPERIMENTAL RESULTS

The minimal teratogenic dose (MTD) [2], i.e., the dose in which the preparation gave a reproducible, minimal teratogenic effect, was 800 mg/kg for carbutamide, when its administration on the 10th day of pregnancy caused malformations in 3.4% of embryos. The MTD for sodium salicylate was 300 mg/kg.

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TABLE 1. Action of Carbutamide and Sodium Salicylate on Embryogenesis of Rats (Administered on the 10th Day of Pregnancy)

Carbutamide (in mg/kg)	Salicylate (in mg/kg)	Glucose	Number of rats in group	No. of implantation sites	Number of dead embryos		Number of living embryos		
					absolute	%	total	malformed	
								absolute	%
800	300	+	9	83	25	30,1±5,0	58	24	41,4±6,5
800	200	+	7	68	34	50,0±6,1	34	20	58,8±8,4
800	25	+	9	91	6	6,6±2,6	85	3	3,5±2,0
600	200	—	6	61	42	68,8±5,9	19	7	36,8±11,1
600	100	—	9	81	13	16,0±4,1	68	2	2,9±2,0
600	50	—	9	92	9	9,8±3,1	83	4	4,8±2,3
400	100	—	9	79	20	25,3±4,9	59	5	8,4±3,6
100	300	—	7	56	25	44,6±6,6	31	2	6,5±4,4
800	300[1]	+	9	89	38	40,4±5,1	51	14	27,4±6,2
600	200[1]	—	6	52	15	28,8±6,3	37	4	10,8±5,0
600	100[1]	—	7	55	11	20,0±5,4	44	1	2,3±2,2
800[1]	300	+	7	72	11	15,3±4,1	61	29	46,0±6,4
800	—	—	6	60	1	1,8±1,7	59	2	3,4±2,4
600	—	—	6	52	1	1,9—1,9	51	—	—
—	300	—	12	126	10	7,9±2,4	116	8	6,9±2,4
—	200	—	11	99	9	9,1±2,9	90	—	—
Control			18	161	9	5,6±1,8	152	—	—
Solvent (Tween)			12	125	8	6,4±2,3	113	—	—

[1] The compound was injected 3 h later

TABLE 2. Teratogenic and Lethal Action of Carbutamide in a Dose of 3000 mg/kg with Supplementary Administration of Glucose

Day of pregnancy	Glucose (in a dose of 6 g per rat)	Number of rats in group	Number of implantation sites	Number of dead embryos		Number of living embryos		
				absolute	%	total	malformed	
							absolute	%
7-th	—	13	125	88	70,4±4,1	37	2	5,4±3,6
7-th	+	14	136	82	60,3±4,2	54	3	5,6±3,1
10-th	—	13	108	46	42,6±4,8	62	62	100,0
10-th	+	18	166	130	78,3±3,2	36	36	100,0

If both compounds were given together in minimal teratogenic doses, 100% of the female rats died within 4-6 h after hypoglycemic convulsions. Accordingly, during the further study of the combined action of the two compounds, a saturated solution of glucose was given in a total dose of 6 g per rat.

Simultaneous adminstration of the compounds (Table 1) led to a sharp increase in the total pathogenic effect: 30.1% of the embryos died and 41.4% showed developmental anomalies.

If the dose of sodium salicylate was reduced to 200 mg/kg, its harmful action was unchanged. However, in a dose of 25 mg/kg, salicylate did not potentiate the harmful action of carbutamide. If the dose of both compounds was simultaneously reduced, the teratogenic effect was diminished, although carbutamide in a dose of 400 km/kg and salicylate in a dose of 100 mg/kg produced developmental anomalies (in 8.4% of cases). If carbutamide was given in a dose of 100 mg/kg and salicylate in a dose of 300 mg/kg, no increase in the teratogenic action of salicylate was observed, i.e., the percentage of malformed embryos in this series was the same as when sodium salicylate was given alone in a dose of 300 mg/kg.

The pathogenic action of carbutamide and sodium salicylate on the embryogenesis of rats thus exhibits a well marked synergism.

If the compounds were not given simultaneously, i.e., carbutamide was injeced 3 h before the salicylate, the postimplantation mortality of the embryos was increased. If salicylate was given first and car-

TABLE 3. Action of Carbutamide and Sodium Salicylate on Blood Sugar Level in % of Initial Blood Sugar)

Agent	Dose (in mg/kg)	No. of rats in group	Mean blood sugar level (in mg%)				Mean decrease (in %)	Mean increase (in %)
			before administration	after administration				
				3 h	6 h	9 h		
Carbutamide	3 000	10	83,6	83,5	108,8	119,5		24,1±5,1
»	800	6	80,3	71,7	50,0	65,8	21,1±4,8	
Salicylate	300	6	100,0	104,5	90,7	93,0	3,1±2,7	
»	600	6	93,7	72,7	72,8	87,7	15,6±5,3	
Carbutamide + Salicylate	800 + 300	6	104,7	67,8	60,0	59,8	40,5±5,2	

butamide later, the teratogenic effect was just as great as when the preparations were given simultaneously (see Table 1).

Table 2 shows that the teratogenic action of carbutamide in a dose of 3000 mg/kg on both the 7th and the 10th days of pregnancy remained unchanged by the administration of large doses of glucose, whereas the lethal effect of the compound on the 10th day was increased. Consequently, large doses of glucose not only did not reduce the teratogenic effect of carbutamide, but increased its lethal action.

To determine whether the harmful action of these two compounds is connected with their hypoglycemic activity, the combined hypoglycemic action of carbutamide and salicylate was studied. The arithmetical changes (decrease or increase) in the blood sugar concentration were therefore determined 3, 6, and 9 h after administration of the preparations and its value expressed as a percentage of its initial level before administration of the pharmacological agents (Table 3).

It is interesting to note that in a high dose (3000 mg/kg) carbutamide not only gave no hypoglycemic effect, but actually raised the blood sugar level by 24.1%. After the combined administration of carbutamide in a dose of 800 mg/kg and salicylate in a dose of 300 mg/kg, the hypoglycemic activity was much stronger than after administration of carbutamide alone. The difference between the action of carbutamide alone and in combination with salicylate is statistically significant. The blood sugar level was lowered by 40.5% for 9 h (see Table 3).

The results of these experiments thus confirmed that the antidiabetic sulfonamides in large doses do not produce hypoglycemia but, on the contrary, raise the blood sugar level [4-6, 7]. This effect is probably related to the action of the compound on the adrenal medulla: causing degranulation of the adrenalin-forming P cells, enlargement of their nuclei, and the secretion of an increased amount of adrenalin [5].

Following the combined administration of carbutamide and salicylate, the hypoglycemic effect is considerably increased, and if certain doses are given hypoglycemic coma with severe convulsions may supervene. This picture is never seen when the sulfonamide alone is given [13].

Synergism in the action of these preparations is also seen when their effect on embryogenesis is studied. Salicylate intensifies the teratogenic action of insulin on chick embryos; this has been attributed to the dissociating action of the preparations on oxidative phosphorylation [14].

The results of the present experiments show that the teratogenic action of carbutamide is not associated with the lowering of the maternal blood sugar level. In doses giving a hypoglycemic action, the compound had no teratogenic activity or it was very slight. The maximal teratogenic effect was given by doses of carbutamide so high that they caused an increase in the blood sugar concentration. This is probably why large doses of glucose did not reduce the teratogenic effect of carbutamide. The teratogenic activity of this sulfonamide may perhaps be related to its dissociating action on oxidative phosphorylation. In this respect the mechanism of the teratogenic action of carbutamide and of sodium salicylate may be similar. This hypothesis requires experimental verification.

The marked synergism in the pathogenic action of carbutamide and sodium salicylate must be taken into consideration when these preparations are administered to pregnant women.

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