The Effects of Cocaine in the Production of Cardiovascular Anomalies in β-Adrenoreceptor Stimulated Chick Embryos[1]

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Summary. Cocaine potentiates the effects of epinephrine and norepinephrine in terms of aortic arch malformation in embryonic chicks. Cocaine itself induces aortic arch malformations when administered in large doses.

In a recent paper it was demonstrated that catecholamines induce cardiovascular abnormalities in embryonic chicks[2]. There is also evidence suggesting that epinephrine and norepinephrine are synthesized[3] and taken up[4] by the chick embryo as early as the second day of development. However, catecholamines are concentrated in embryonic chick hearts only after the 5th day of development, sympathetic innervation having been completed by this time[5]. Cocaine is known to produce an exaggerated responsiveness to neurohumors by presumably preventing their uptake into nerve endings[6–9]. Such a phenomenon has also been demonstrated in embryonic chicks[4,5] and probably allows sympathomimetic amines to persist near receptors of effector organs in higher concentrations for a longer period of time. This communication describes studies in which embryonic chicks were pretreated with cocaine prior to administration of four sympathomimetic amines.

White Leghorn eggs were incubated and prepared for the administration of experimental compounds using the materials and methods described previously[10].

When embryos had developed to HAMBURGER-HAMILTON[11] stage 26 of embryogenesis, they were pretreated with 5×10^{-7} *M* cocaine hydrochloride in 5 µl 0.9% saline solution. These injections occurred 4 to 5 min prior to administrations of equal volumes of sympathomimetic amines dissolved in saline. Doses of 4×10^{-9} *M* isoproterenol (I), norepinephrine (N) and phenylephrine (P) were utilized in this study. Only 5×10^{-10} *M* epinephrine (E) was used since doses greater than this together with cocaine resulted in extremely high mortality rates within hours following injections. The hydrochloride salts of l-isomers were used. All experiments were conducted in a controlled environment with temperatures ranging from 37.0 °C to 38.0 °C. Injections and dissections were performed by methods previously documented[2]. Control embryos were administered 10 µl 0.9% saline.

The data in the Table demonstrate that 1. cocaine potentiates the effects of epinephrine and norepinephrine in terms of increased cardiovascular anomaly rates but has little effect with isoproterenol or phenylephrine; and 2. that the order of potency of the 4 drugs (I > E > N ⩾ P) in producing one specific type of aortic arch anomaly (IA-1)[2] suggests that β-receptor stimulation induces aortic arch anomalies in embryonic chicks.

Analyses of data indicate that epinephrine and norepinephrine induce more than 10 times as many anomalies when injected with cocaine as they do when injected

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[2] R. J. HODACH, A. E. HODACH, J. F. FALLON, J. D. FOLTS, H. J. BRUYÈRE and E. F. GILBERT, Teratology *12*, 33 (1975).
[3] L. J. IGNARRO and F. E. SHIDEMAN, J. Pharmac. exp. Ther. *159*, 38 (1968).
[4] L. J. IGNARRO and F. E. SHIDEMAN, J. Pharmac. exp. Ther. *159*, 49 (1968).
[5] L. J. IGNARRO and F. E. SHIDEMAN, J. Pharmac. exp. Ther. *159*, 59 (1968).
[6] J. AXELROD, H. WEIL-MALHERBE and R. TOMCHICK, J. Pharmac. exp. Ther. *127*, 251 (1959).
[7] L. G. WHITBY, J. AXELROD and H. WEIL-MALHERBE, J. Pharmac. exp. Ther. *132*, 193 (1961)
[8] G. HERTTING, J. AXELROD and L. G. WHITBY, J. Pharmac. exp. Ther. *134*, 146 (1961).
[9] E. MUSCHOLL, Br. J. Pharmac. Chemother. *16*, 352 (1961).
[10] R. J. HODACH, E. F. GILBERT and J. F. FALLON, Teratology *9*, 203 (1974).
[11] V. HAMBURGER and H. L. HAMILTON, J. Morph. *88*, 49 (1951).

Effects of cocaine pretreatment on aortic arch anomaly rates in sympathomimetic amine treated chick embryos

Dose (*M*)/Drug	Frequency of aortic arch anomalies[a]	%	Theoretical range, agents acting independently (%)	Rate of IA-1 anomalies	%	Rate of aortic hypoplasia/interrupted aortic arch complexes	%
4×10^{-9} Isoproterenol	14/52	25		6/53	11	8/53	15
4×10^{-9} Epinephrine	5/47	9		3/47	6	2/47	4
5×10^{-10} Epinephrine	0/27	0		0/27	0	0/27	0
4×10^{-9} Norepinephrine	2/87	0		2/87	2	0/87	0
4×10^{-9} Phenylephrine	3/101	1		1/101	1	0/101	0
5×10^{-7} Cocaine	6/97	4		3/81	4	0/81	0
4×10^{-9} Isoproterenol[b]	17/57	28	25–29	8/57	14	2/57	4
5×10^{-10} Epinephrine[b]	5/28	16	Approximately 4	5/28	18	0/28	0
4×10^{-9} Norepinephrine[b]	13/71	16	Approximately 4	10/71	14	0/71	0
4×10^{-9} Phenylephrine[b]	2/30	5	4–5	2/30	7	0/30	0
Controls	3/175	0		2/175	1	0/175	0

[a] Values in second column here represent experimentally derived frequencies minus 2% due to spontaneous anomaly occurrences.
[b] Compounds were administered 4 to 5 min subsequent to treatment with 5×10^{-7} *M* cocaine.

alone. The potentiated anomaly rates may possibly be explained by inhibited catecholamine uptake with the use of cocaine. These results agree with a study of catecholamine uptake in embryonic chicks using tritiated norepinephrine and epinephrine[4]. Two enzymes which metabolize catecholamines (catechol-*O*-methyl transferase – COMT and monoamine oxidase – MAO) are present in the chick embryo on the 4th day of incubation[12]. However, MAO is intracellular and probably does not metabolize the exogenous norepinephrine and epinephrine. The extent to which COMT metabolizes catecholamines is most likely insignificant in the termination of overall catecholamine activity[4].

Cocaine does not increase the anomaly rate among isoproterenol treated embryos. This result may possibly be explained by the fact that isoproterenol is apparently not taken up by post-ganglionic sympathetic neurons[13]. However, cocaine in combination with isoproterenol induces different types of anomalies from those frequently induced by isoproterenol alone, that is, less cases of aortic hypoplasia and interrupted aortic arch complexes were observed.

Furthermore, cocaine does not increase the anomaly rate among those embryos treated with phenylephrine. Assuming that cardiovascular anomalies can be induced in embryonic chicks by β-adrenoreceptor stimulation, this result is expected. Phenylephrine acts primarily on α-adrenergic receptors.

This study has also shown that, using as a criterion for potency the frequency of aortic arch anomaly type IA-1 (formerly described as absence of the 3rd right aortic arch with an anomalous origin of the right common carotid artery from the right ductus caroticus)[2], I is more potent than E which in turn is more potent than N or P at 4×10^{-9} *M*/embryo. This relation of I $\gg$ E $>$ N or P correlates with a β-adrenoreceptor response to the drugs and confirms previous findings that a β-receptor mechanism might possibly be involved in the induction of cardiovascular anomalies[2].

Furthermore, cocaine specifically potentiates the effects of epinephrine and norepinephrine in the formation of aortic arch anomaly type IA-1; from 0 to 14% in the case of epinephrine (considering IA-1 anomalies induced spontaneously and with cocaine) and from 1 to 10% in the case of norepinephrine (computed from Table). Cocaine does not potentiate the IA-1 anomaly rate when administered with isoproterenol or phenylephrine. Cocaine in all cases did not potentiate aortic hypoplasia or interrupted aortic arch complexes.

Cocaine in small doses (5×10^{-9} to 1×10^{-7} *M*) does not induce aortic arch or cardiac anomalies in embryonic chicks. However, type IA-1 is occasionally induced when 5×10^{-7} *M* is administered (see Table). Furthermore, several cases were observed which demonstrated premature closure of the right ductus arteriosus and persistence of the left ductus caroticus. This may be explained if cocaine also affects the reuptake of endogenous catecholamines into nerve endings. In effect large amounts of circulating endogenous catecholamines might possibly cause cardiovascular anomalies in this system. Since it has been demonstrated that cocaine in a dose $^1/_7$ to $^1/_6$ that used in this study inhibits more than 80% of norepinephrine uptake by the whole chick embryo and more than 50% by the embryonic heart on the 5th day of development[4], this hypothesis seems possible.

In conclusion, whereas cocaine was administered to embryos 3 h prior to catecholamine injection in a previous study[4], this study demonstrated potentiation with a 5 min pretreatment period. This finding suggests that cocaine rapidly affects the uptake mechanism of nerve endings in embryonic chicks.

[12] L. J. Ignarro and F. E. Shideman, J. Pharmac. exp. Ther. *159*, 29 (1968).

[13] G. Hertting, Biochem. Pharmac. *13*, 1119 (1964).

A New Quinoline-Carbamate Aphicide[1]

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Summary. We found that it was nearly impossible to apply the quinoline-carbamate aphicide per os by means of synthetic diets, owing to its high feeding-deterrent-effect. After application via the roots of the host plant, this systemic compound is deposited on the leaf surface. The results suggest that the toxic effect is not the result of the oral uptake of phloem sap, but of the tarsal contact with the toxicant. Sensitivity of aphids to this compound and LD_{50}-values were determined after topical applications.

The aphicidal effect and the symptoms and phases of the intoxication of a new aphicide were investigated. Earlier experiments from the producer had shown that the compound was much more effective against aphids than against other insects. The following aphid species were used: *Aulacorthum circumflexum* (Buckton), *Aphis craccivora* Koch and two strains of *Myzus persicae* (Sulzer), one susceptible to insecticides and the second resistant to organophosphorus compounds. All aphids were reared parthenogenetically on suitable host plants in the laboratory. The experiments with oral application were carried out only with *A. circumflexum* since it has a spontaneous and even nutrient uptake from artificial media[4,5].

The aphicide, (concentration 99.9%) a quinoline-carbamate[6], 4 (N.N-dimethyl-carbamoyloxy-)-2-methyl-5.6.7.8-tetrahydro-quinoline, also known as Hoe 25 682,

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[4] P. Ehrhardt, Habilitationsschrift, Universität Bonn (1968).

[5] D. Holtgräwe and W. Kloft, Oecologia *14*, 229 (1974).

[6] W. Knauf, G. Salbeck and F. Schwerdtle, Meded. Landbhoogesch. Opzoek Stns Gent *27*, 427 (1975).