2 Baker, P.F., Blaustein, M.P., Keynes, R.D., Manil, J., Shaw, T.I., and Steinhardt, R.A., J. Physiol., Lond. *200* (1969) 459.
3 Hougen, T.J., and Smith, T.W., Circulation Res. *42* (1978) 856.
4 Langer, G.A., and Serena, S.D., J. molec. cell. Cardiol. *1* (1970) 65.
5 Lee, C.O., Kang, D.H., Sokol, J.H., and Lee, K.S., Biophys. J. *29* (1980) 65.
6 Eisner, D.A., Lederer, W.J., and Vaughn-Jones, R.D., J. Physiol., Lond. *317* (1981) 189.
7 Repke, K., *in:* Drugs and Enzymes, vol.4, p.65. Proc. Int. Pharmac. Meeting Prague, Pergamon Press, New York 1965.
8 Tuttle, R.S., Wit, P.N., and Farah, A.J., Pharmac. exp. Ther. *169* (1961) 287.
9 Ghysel-Burton, J., and Godfraind, T., J. Physiol., Lond. *266* (1977) 75P.
10 Ellis, D., J. Physiol., Lond. *273* (1977) 211–240.
11 Baker, P.F., and Willis, J.S., J. Physiol., Lond. *224* (1972) 463.
12 Noble, D., Cardiovasc. Res. *14* (1980) 495.
13 Vassalle, M., Circulation Res. *27* (1970) 361.
14 Eisner, D.A., and Lederer, J., J. Physiol., Lond. *303* (1980) 414.
15 Gadsby, D., and Cranefield, P.F., J. gen. Physiol. *73* (1979) 819.
16 Hoffman, B.F., in: Basic and Clinical Pharmacology of Digitalis, p.118. Thomas, Springfield 1972.
17 Haas, H.G., and Kern, R., Pflügers Arch. *291* (1966) 69.
18 Vereecke, J., Isenberg, G., and Carmeliet, E., Pflügers Arch. *384* (1980) 207.
19 Attwell, D., Cohen, I., Eisner, D.A., Ohba, M., and Ojeda, C., Pflügers Arch. *379* (1979) 137.
20 Cohen, I., and Kline, R.P., Circulation Res. *50* (1982) 1.
21 Hodgkin, A.L., and Katz, B., J. Physiol., Lond. *108* (1949) 37.
22 Jack, J.J.B., in: The Peripheral Nerve, p.740. Halsted, London 1976.

0014-4754/83/111280-03$1.50+0.20/0

Cholecystokinin-octapeptide-induced hyperthermia in guinea-pigs

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Summary. Intracerebroventricular administration of cholecystokinin-octapeptide (CCK-8) at room temperature (21 °C) induced dose-related hyperthermia in guinea-pigs and also produced hyperthermia at low (10 °C) and high (30 °C) ambient temperatures. CCK-8-induced hyperthermia was direct and not mediated by prostaglandins, norepinephrine, cyclic AMP or naloxone-sensitive receptors.

Cholecystokinin (CCK) has been shown to be present in the central nervous system of several species[1-4]. Several groups[5-7] have reported CCK-immunoreactive neuronal structures in the hypothalamus. CCK has been implicated in the neuroendocrine control of thyroid stimulating hormone (TSH), luteinizing hormone (LH) and prolactin[8], as well as in the regulation of feeding[9] and thermoregulation[10,11]. Cholecystokinin octapeptide (CCK-8) induces hypothermia in rats[10] and mice[11]. The present study was undertaken in guinea-pigs to: a) determine the effect on temperature of CCK-8 at different temperatures; b) ascertain whether prostaglandins (PG), cAMP or norepinephrine (NE) are involved in CCK-8-induced hyperthermia since each of these has been found to be hyperthermic in guinea-pigs; and c) find out the effect of naloxone on CCK-8-induced hyperthermia.

Materials and methods. Drugs used. Indomethacin and theophylline (Sigma Chemical Company, St.Louis, Mo.); naloxone hydrochloride (National Institute on Drug Abuse, Washington, D.C.); cholecystokinin octapeptide (Peninsula Laboratories, San Carlos, Ca.); phenoxybenzamine (Smith, Kline and French Laboratory, Philadelphia, Pa.); ketamine hydrochloride (Parke-Davis, Detroit, Mi.); xylazine (Haver-Lockhart, Shawnee, Ka.); acepromazine (Ayerst Laboratories Inc., New York, N.Y.).

Methods. Male guinea-pigs of the Hartley strain weighing 250–300 g were anesthetized with 1 ml/kg (i.m.) of a mixture of ketamine 50 mg/kg, xylazine 5 mg/kg and acepromazine 1 mg/kg, and were placed in a guinea-pig head holder (David Kopf Instruments, Tujunga, Ca.). After exposure and the retraction of the superior sagittal sinus, a single cannula was inserted into a lateral ventricle according to the coordinates: (derived from the atlas of Luparello et al.[12]) 2 mm lateral to the midline and 0.5 mm rostral to the bregma, vertically until cerebrospinal fluid rose in the cannula. Dental acrylic was used to secure the cannula. Injection sites were verified post mortem. The volume of injection was always 10 µl. At least 1 week was allowed for recovery before the animals were used for experiments. Animals used once were randomly used again only one more time but after a 7-day period. Injections were made at the same time of day (10.00 h ± 6 min) to avoid diurnal variation in temperature.

CCK-8, naloxone and theophylline were dissolved in sterile, non-pyrogenic 0.9% saline. Phenoxybenzamine was dissolved in 10% dimethyl sulfoxide and pyrogen-free distilled water while indomethacin was dissolved in a mixture of 1% NaOH and sterile non-pyrogenic distilled water. For

Table 1. Hyperthermic effect of i.c.v. administration of cholecystokinin (CCK-8) in guinea-pigs

Treatment	Hyperthermia (°C, mean ± SE)
Saline 0.9%	0.1 ± 0.05 (n = 10)
CCK-8 10 µg	0.5 ± 0.15 (n = 5)*
CCK-8 20 µg	0.9 ± 0.08 (n = 15)*
CCK-8 50 µg	1.3 ± 0.18 (n = 5)*
CCK-8 100 µg	1.5 ± 0.24 (n = 5)*

* Significantly different from 0.9% saline value $p < 0.05$.

Table 2. Hyperthermic effect of CCK-8 (20 µg, i.c.v.) at different ambient temperatures

Ambient temperature (°C)	Hyperthermia (°C, mean ± SE)
10	0.7 ± 0.18 (n = 5)
21	0.9 ± 0.08 (n = 15)
30	1.2 ± 0.22 (n = 5)

our study doses of antagonists/inhibitors which by themselves had no significant effect on temperature were selected either from our previous experiments or in pilot experiments.

The animals were placed in restraining cages 2 h before the beginning of experiments carried out at an environmental temperature of 21 ± 1 °C. Body temperatures were measured with copper constantan thermocouples inserted approximately 6 cm into the rectum and connected to an Autodata Nine Analog/Digital Data acquisition system (Acurex Corp., Ca.). Statistical significance was assessed with Student's t-test and a significance of $p < 0.05$ was considered significant.

Results. Effects of intracerebroventricular (i.c.v.) administration of CCK-8 in control animals: 10–50 µg of CCK-8-induced a dose-related hyperthermia (table 1) and doses smaller than 10 µg did not produce any significant change in temperature. Similar injection of the vehicle used to dissolve CCK-8 did not cause any significant change in temperature (table 1). No significant differences were observed between the temperature responses to 50 and 100 µg of CCK-8. 300 µg of CCK-8 induced a fall (0.8 ± 0.18, n = 5) in temperature. Unless otherwise mentioned 20 µg of CCK-8 was used as a test dose throughout our experiments. Also, CCK-8-induced hyperthermia at ambient temperatures of 10 °C and 30 °C (table 2).

Effect of indomethacin and phenoxybenzamine on CCK-8-induced hyperthermia: In control animals, 1–5 mg/kg (i.p.) of indomethacin or phenoxybenzamine (10–50 µg, i.c.v.) did not alter body temperature and did not antagonize hyperthermia induced by CCK-8 (table 3). The vehicles used to dissolve the indomethacin and phenoxybenzamine caused a 0.15 ± 0.05 °C rise (n = 5) and a 0.15 ± 0.08 °C fall in temperature (n = 5) respectively.

Effect of theophylline and naloxone on CCK-8-induced hyperthermia: Variable doses of theophylline (10–30 µg, i.c.v.) did not accentuate CCK-8-induced hyperthermia (table 3). Pretreatment with naloxone (1–5 mg/kg, i.p.) had no significant effect on body temperature in control animals and did not attenuate the hyperthermia due to CCK-8 (table 3).

Table 3. Effects of various pretreatments on hyperthermia induced by CCK-8 (20 µg, i.c.v.) in guinea-pigs

Treatment	Hyperthermia (°C, mean ± SE)
Saline 0.9% + CCK-8	0.9 ± 0.08 (n = 15)
Vehicle + CCK-8	1.0 ± 0.15 (n = 10)
Indomethacin (1 mg/kg, i.p.) + 0.9% saline	0.15 ± 0.12 (n = 10)
Indomethacin (3 mg/kg, i.p.) + 0.9% saline	0.15 ± 0.08 (n = 11)
Indomethacin (5 mg/kg, i.p.) + 0.9% saline	− 0.20 ± 0.15 (n = 10)
Indomethacin (1 mg/kg, i.p.) + CCK-8	1.0 ± 0.15 (n = 5)
Indomethacin (3 mg/kg, i.p.) + CCK-8	1.1 ± 0.10 (n = 5)
Indomethacin (5 mg/kg, i.p.) + CCK-8	0.7 ± 0.20 (n = 5)
Phenoxybenzamine (10 µg, i.c.v.) + 0.9% saline	− 0.1 ± 0.08 (n = 11)
Phenoxybenzamine (30 µg, i.c.v.) + 0.9% saline	− 0.1 ± 0.10 (n = 12)
Phenoxybenzamine (50 µg, i.c.v.) + 0.9% saline	− 0.20 ± 0.15 (n = 10)
Phenoxybenzamine (10 µg, i.c.v.) + CCK-8	0.9 ± 0.15 (n = 5)
Phenoxybenzamine (30 µg, i.c.v.) + CCK-8	0.8 ± 0.10 (n = 5)
Phenoxybenzamine (50 µg, i.c.v.) + CCK-8	0.7 ± 0.25 (n = 5)
Theophylline (10 µg, i.c.v.) + 0.9% saline	0.15 ± 0.10 (n = 12)
Theophylline (30 µg, i.c.v.) + 0.9% saline	0.20 ± 0.12 (n = 12)
Theophylline (10 µg, i.c.v.) + CCK-8	1.10 ± 0.18 (n = 5)
Theophylline (30 µg, i.c.v.) + CCK-8	1.30 ± 0.22 (n = 5)
Naloxone (1 mg/kg, i.p.) + 0.9% saline	0.10 ± 0.06 (n = 11)
Naloxone (3 mg/kg, i.p.) + 0.9% saline	− 0.10 ± 0.15 (n = 10)
Naloxone (5 mg/kg, i.p.) + 0.9% saline	− 0.20 ± 0.12 (n = 12)
Naloxone (1 mg/kg, i.p.) + CCK-8	1.10 ± 0.20 (n = 5)
Naloxone (3 mg/kg, i.p.) + CCK-8	1.0 ± 0.12 (n = 5)
Naloxone (5 mg/kg, i.p.) + CCK-8	0.8 ± 0.14 (n = 5)

Discussion. When injected into the lateral cerebral ventricle of guinea-pigs CCK-8 resulted in a dose-dependant hyperthermia although there are reports of hypothermia when CCK-8 was injected intraventricularly in rats[10] and subcutaneously in mice[11]. The hyperthermic effect of CCK-8 was obtained not only at room temperature, but also at low and high ambient temperatures. Doses smaller than 10 µg did not produce any significant change in temperature and doses larger than 100 µg induced hypothermia. At the present time there is no explanation for the lack of effect of CCK-8 in small doses and hypothermia induced by large doses. However, it is suggested that the lack of effect due to small doses may be due to rapid metabolism of CCK-8 and species variation and that the hypothermia induced by large doses may be due to a non-specific depressant effect. Similar results have been reported in guinea-pigs for cAMP[13] and opioid peptides such as β-endorphin, methionine-enkephalin and leucine-enkephalin[14]. The presence of CCK in the hypothalamus (the region believed to contain the primary temperature control in homeotherms), and the regulated rise of temperature at different ambient temperatures suggest that it may play an important physiological role in thermoregulation.

In guinea-pigs, prostaglandins, norepinephrine and cAMP induce fever[15–17]. Our results indicate that CCK-8-induced hyperthermia was not mediated through PG since a PG synthesis inhibitor, indomethacin did not attenuate it. Lack of inhibition by indomethacin also demonstrates that the hyperthermic responses to CCK-8 was not due to contamination by pyrogen since the same dose of indomethacin very quickly abolishes fever following endotoxin administration (unpublished data). NE-induced hyperthermia in guinea-pigs is selectively antagonized by an α-adrenergic blocker, phentolamine[18]. Phenoxybenzamine did not alter the hyperthermia due to CCK-8 indicating that NE is not involved in this hyperthermia. NE, serotonin and PG are widely accepted as being involved in temperature regulation and are known to stimulate cAMP formation in brain tissue[19–21]. cAMP could be the terminal link in bacterial endotoxin, PGE_1 and NE fever in the rabbit since both of these types of fever are accentuated by theophylline[22,23]. It is suggested that a NE-cAMP link occurs in the hypothalamic pathways which mediate fever induced by endotoxin and PGE_2 in the rat[24]. In our experiments, however theophylline did not accentuate CCK-8-induced hyperthermia indicating that this hyperthermia may not be mediated by cAMP.

The hyperthermic effect of morphine and β-endorphin were readily antagonized by naloxone in guinea-pigs[14] which suggests that this hyperthermia is mediated through naloxone-sensitive receptors. Similar findings have been reported in other species too[25]. The failure of naloxone to attenuate CCK-8-induced hyperthermia suggests that naloxone-sensitive receptors were not involved in this hyperthermia.

From the present results it would appear that the CCK-8-induced hyperthermia is direct and that it is not mediated by PG, NE, cAMP or naloxone-sensitive receptors. CCK-8-induced hyperthermia was obtained in guinea-pigs and that this is not a general finding.

1 Dockeray, G.J., Nature *264* (1976) 568.
2 Muller, J.E., Strauss, E., and Yalow, R.S., Proc. natl Acad. Sci. USA *74* (1977) 3035.
3 Strauss, E., and Yalow, R.S., Proc. natl Acad. Sci. USA *75* (1978) 486.

4 Vanderhaeghen, J.J., Signeau, J.C., and Gepts, W., Nature *257* (1975) 604.
5 Innes, R., Correa, F.M., Uhl, G., Schneider, B., and Snyder, S.H., Proc. natl Acad. Sci. USA *76* (1979) 521.
6 Loren, I., Alumets, J., Hakanson, R., and Sundler, F., Histochemistry *59* (1979) 249.
7 Vanderhaeghen, J.J., Lostra, F., De May, J., and Giles, C., Proc. natl Acad. Sci. USA *77* (1979) 1190.
8 Yaksh, T.L., Abay, E.O., and Go, V.L.W., Brain Res. *242* (1982) 279.
9 Della-Fera, M.A., and Baile, C.A., Science *206* (1979) 471.
10 Morley, J.E., Levine, A.S., and Lindblad, S., Eur. J. Pharmac. *74* (1981) 249.
11 Zetler, G., Neuropharmacology *21* (1982) 795.
12 Luparello, T.J., Stein, M., and Park, C.D., J. comp. Neurol. *122* (1964) 201.
13 Kandasamy, S.B., and Williams, B.A., Neuropharmacology *22* (1983) 65.
14 Kandasamy, S.B., and Williams, B.A., Neuropharmacology *22* (1983) 621.
15 Szekely, M., and Komaromi, I., Acta physiol. hung. *51* (1978) 293.
16 Komaromi, I., Acta physiol. hung. *47* (1976) 29.
17 Kandasamy, S.B., Kirlan, W.G., and Kaul, P.N., Life Sci. *28* (1981) 2553.
18 Komaromi, I., Experientia *33* (1977) 1083.
19 Kakiuchi, S., and Rall, T.W., Molec. Pharmac. *4* (1968) 367.
20 Klainer, L.M., Chi, Y.M., Freidberg, S.L., Rall, T.W., and Sutherland, E.W., J. biol. Chem. *237* (1962) 1239.
21 Zor, U., Kaneko, T., Schneider, H.P.G., McCann, S.M., Lowe, I.P., Bloom, G., Borland, B., and Field, J.B., Proc. natl Acad. Sci. USA *63* (1969) 918.
22 Woolf, C.J., Willies, G.H., Laburn, H.P., and Rosendorff, C., Neuropharmacology *14* (1975) 397.
23 Kandasamy, S.B., Pharmacology *20* (1980) 304.
24 Lin, M.T., Wu, J.J., Chandra, A., and Tsay, B.L., J. Pharmac. exp. Ther. *222* (1982) 251.
25 Clark, W.G., Fedn Proc. *40* (1981) 2754.

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Negative inotropic effect of some H_2-receptor antagonists on the isolated human atria[1]

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Summary. H_2-Receptor antagonists were found to possess in various degrees a negative inotropic effect on human atria in vitro. This effect seemed to be independent of H_2-receptor blockade and, at least in the case of oxmetidine, seemed to involve calcium ion transport and/or utilization.

It is well known that histamine possesses a positive inotropic effect on the heart of different species, mediated through excitation of H_2-receptors. Thus, H_2-antagonists could be supposed to possess a negative inotropic effect on the heart. However, in spite of the widespread clinical use of such compounds, reports of any kind of activity on the heart are very few and concern an exceedingly small (less than 0.01%) percentage of subjects[3-5]. This apparent discrepancy may result from the relatively small number of histamine receptors in the heart or the degree of excitation of these receptors which, in physiological conditions, is completely overwhelmed by the excitation of the beta-adrenergic receptors. It is probable that those cases in which some negative effects on the heart following the administration of H_2-blockers were noted were characterized by abnormally high levels of histamine, which contributed consistently to the maintenance of cardiac activity. On the other hand, nonspecific effects of the H_2-antagonists which are known to be present in different tissues, may be responsible for some negative effects of these drugs on the human heart[6]. In the present study, we investigated the action of some new H_2-antagonists of different structure on human isolated heart preparations removed during surgery.

Material and methods. Right atria biopsy samples (1×0.2 cm) obtained during cardiac surgery were used. The technique described by Gristwood et al.[7] was followed. After removal of the tissue it was immediately placed at 4 °C in oxygenated Krebs solution (mM: NaCl 113; $NaHCO_3$ 2.5; KCl 4.7; $CaCl_2$ 1.9; KH_2PO_4 1.2; $MgSO_4$ 1.2; glucose 11.5) and mounted vertically in a 10 ml organ bath at 37 °C. Two platinum electrodes were used to drive electrically the tissue by square wave pulses of 2 msec duration, frequency of 1 Hz and twice threshold voltage. The tissues were allowed to equilibrate at a tension of 1 g for about 60 min. Contractions were recorded by a transducer and a microdynamometer. When the electrically-stimulated contractions were too small, the effect of the compounds was tested against contractions obtained by administering $CaCl_2$ in contractions varying from 0.5 to 3×10^{-3} M.

Drugs. Compounds used were: cimetidine, oxmetidine and compound marked SKF 93479 (kindly supplied by the SKF, Welwyn, England), ranitidine (Glaxo), tiotidine and propranolol (ICI), compound marked DA 4577 4(5)-(4-isopropylaminomethyleniminophenyl)imidazole (De Angeli, Milano, Italy), verapamil (Knoll) and procaine (Fluka).

Results. In our experimental conditions there was no correlation between calcium concentration of the medium and mean contractile force of cardiac biopsy samples. The amount of calcium added to the nutrient fluid was adjusted for each individual preparation in order to obtain a constant contractile response under control conditions.

The mean value of the atrial contraction after calcium administration was 0.85 ± 0.04 g ($n = 35$). In these conditions the effects of the H_2-antagonists varied considerably with the different molecules: cimetidine and ranitidine were virtually ineffective even at the maximum concentration tested (10^{-3} M). At this concentration, tiotidine, which is more potent for its specific effect on the H_2-receptors, showed a remarkable negative inotropic effect. The new compound DA 4577 which again has been shown to be more potent than cimetidine and ranitidine as an H_2-blocker[9], was virtually ineffective in high concentrations (10^{-4} M) and showed a slight negative inotropic effect only at $1\text{-}3 \times 10^{-3}$ M. Conversely oxmetidine and SKF 93479 had a consistent dose-dependent inhibitory effect from