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PRELIMINARY COMMUNICATION

ENKEPHALIN- AND SUBSTANCE P-LIKE IMMUNOREACTIVITIES OF MAMMALIAN SPERM AND ACCESSORY SEX GLANDS

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Acetylcholine (ACh) occurs in mammalian spermatozoa (1-3). Selective inhibitors of choline acetyltransferase (ChA) depress the motility index of human spermatozoa, and there is a positive relationship between the depression of the sperm motility index and inhibition of ChA (4). According to our studies, methionine enkephalin and Substance P regulate ACh release by inhibiting or enhancing Ca^{2+} uptake by the neuronal tissues (5-7). Similarly, methionine enkephalin and Substance P may play a regulatory role in ACh-induced Ca^{2+} movements and, therefore, progressive sperm motility. We have extracted enkephalins and related opioid peptides and Substance P from human, bull, and rat spermatozoa; human seminal plasma; and accessory sex glands of the rat. Our investigations indicate that there are high levels of these peptides in human seminal plasma, possibly from secretions of the epididymis and accessory sex glands.

MATERIALS AND METHODS

Standard methionine enkephalin and leucine enkephalin were purchased from the Pierce Chemical Co., Rockford, IL. Rabbit anti-met enkephalin, anti-leu-enkephalin, anti-β-endorphin, anti-Substance P, [^{125}I]methionine enkephalin, [^{125}I]leucine enkephalin, [^{125}I]-β-endorphin, and [^{125}I]Substance P were supplied by the Immuno Nuclear Corp., Stillwater, MN. Each antibody has 100% cross reactivity with its corresponding antigen-peptide and negligible cross reactivity with other peptides. For example, the antibody of methionine enkephalin has cross reactivity of 100% with standard methionine enkephalin; 1.6% with leucine enkephalin; and 0.002% with Substance P and β-endorphin.

Extraction of peptides from spermatozoa and seminal plasma. The collection of human ejaculates, separation and washing of spermatozoa, source of bull spermatozoa, and collection of rat epididymal spermatozoa have been described in our previous publications (1,4). Seminal vesicles and prostate glands were dissected from rats. All of the above tissues were extracted for peptides by procedures published from our laboratories (8,9). The final preparations were waxy residues which were stored at -20°.

Radioimmunoassays for methionine enkephalin, leucine enkephalin, β-endorphin, and Substance P. Each waxy extract was resuspended in distilled

deionized water (2 ml), warmed in a boiling water bath for 15 min, cooled, and centrifuged at 100,000 g for 15 min. The supernatant fraction was freeze dried and dissolved in an appropriate buffer for radioimmunoassays.

Radioimmunoassays for enkephalins (10,11), β-endorphin (12,13), and Substance P (14) have been described by several authors and by Dr. A. W. Lindell in the pamphlets supplied by the Immuno Nuclear Corp. with the antibodies. The essential features of our assays (8,9) are similar to those described by others (10-14).

Characterization of biological activity of peptides. The biological activity of the extracts was tested on intramurally stimulated rat vas deferens according to the method of Sastry et al. (8,9).

RESULTS AND DISCUSSION

The occurrence of methionine enkephalin, leucine enkephalin, β-endorphin, and Substance P in human spermatozoa was demonstrated by sensitive and selective radioimmunoassays (Table 1). There were higher levels of methionine enkephalin and leucine enkephalin in seminal plasma than in spermatozoa. Higher levels of Substance P were present in sperm cells than in plasma. A small fraction of some peptides might have decomposed during collection and liquefaction of human ejaculates and separation of spermatozoa. The above peptides are stable under the present extraction conditions (8,9). The values for human spermatozoa and semen are approximate and may be slightly lower than the actual values.

In view of the high levels of opioid peptides found in human seminal plasma compared to human spermatozoa, spermatozoa, caudal epididymis, seminal vesicles, and prostate gland from the rat were extracted and analyzed for opioid peptides and Substance P. Caudal epididymis and prostate contained higher levels of methionine enkephalin, leucine enkephalin, β-endorphin and Substance P than spermatozoa (Table 1). There were no significant differences in β-endorphin-like and Substance P-like immunoreactivities in seminal vesicles and spermatozoa of the rat.

Standard Substance P (5 μg/ml) increased the electrically stimulated contraction height of the rat vas deferens preparation (50%). Leucine enkephalin (5 μg/ml) decreased the contraction height of the vas deferens by 25%. Mixtures of Substance P and leucine enkephalin caused biphasic responses in this preparation. There was (a) an initial facilitation followed by inhibition of transmission with mixtures containing higher proportions of Substance P relative to leucine enkephalin, and (b) an initial phase of inhibition followed by facilitation of transmission with mixtures containing higher proportions of leucine enkephalin. The extracts of spermatozoa, seminal plasma, and accessory sex glands produced the following responses: (1) Extracts containing very high immunoreactivities to opioid peptides compared to Substance P (e.g. extract of human seminal plasma) inhibited chemical transmission. (2) Extracts containing very high immunoreactivities to Substance P compared to the opioid peptide (e.g. caudal epididymis) facilitated transmission. (3) All other extracts caused biphasic responses. Both facilitory and inhibitory responses of the extracts are dose-dependent. In all cases, the inhibitory responses were partially antagonized (40-50%) by naloxone (100 nM). Further work is in progress to separate and identify

enkephalins and Substance P from various extracts by high performance liquid chromatography.

Table 1. Immunoreactivities for opioid peptides and Substance P in spermatozoa and accessory sex glands*

Species and Tissue	Methionine enkephalin (pg/mg protein)	Leucine enkephalin (pg/mg protein)	β-Endorphin (pg/mg protein)	Substance P (pg/mg protein)
Human				
Spermatozoa	583 ± 174	1080 ± 650	570 ± 290	2145 ± 661
Seminal plasma	2228 ± 63	>9000+	---+	572 ± 60
Rat				
Spermatozoa	244 ± 80	166 ± 96	68 ± 68	932 ± 33
Caudal epididymis	1300 ± 191	2467 ± 814	1585 ± 486	>15,000
Seminal vesicles	1360 ± 121	9663 ± 1508	56 ± 35	777 ± 113
Prostate	976 ± 307	3246 ± 1188	1176 ± 337	5379 ± 1793

*Values for the extracts were prepared as described in Materials and Methods. Values are means ± S.E. N = 3-4.

+Due to interfering substances, exact values could not be determined.

The actual expulsion of fluids from the epididymis, seminal vesicles and prostate into the prostatic urethra and the progression of the total fluid, under pressure, through the full length of the penile urethra to the urethral meatus is the physiologic expression of the male orgasmic experience. The seminal vesicles, prostate gland and ductus epididymis have cholinergic innervation which may play a significant role in the passage of total fluid. Opioid peptides predominate compared to Substance P in seminal plasma. It is possible that the penile urethra may contain receptors sensitive to opioids. Alternatively, these opioid peptides may antagonize sympathetic activation of the vas deferens and blood vessels in the penis after an orgasm. If the Substance P concentration in ejaculates increases considerably over that of opioid peptides, it may induce uterine cramps in women, because Substance P increases the contractions of smooth muscle. As stated in the introduction, opioid peptides and Substance P may also regulate ACh induced Ca^{2+} movements in spermatozoa. Influx of extracellular Ca^{2+} is essential for sperm motility and acrosome reaction.

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